PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/00, 39/00, 39/44, 39/395, 51/00, C07K 2/00, 4/00, G01N 33/53, 33/543, 33/566

A1

(11) International Publication Number: **WO 99/64037**

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/US99/12620

(22) International Filing Date: 8 June 1999 (08.06.99)

(30) Priority Data:

60/088,448	8 June 1998 (08.06.98)	US
60/093,072	16 July 1998 (16.07.98)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	60/088,448 (CON)
Filed on	8 June 1998 (08.06.98)
US	60/093,072 (CON)
Filed on	16 July 1998 (16.07.98)

(71) Applicant *(for all designated States except US)*: ADVANCED MEDICINE, INC. [US/US]; 280 Utah Avenue, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US). JUDICE, J., Kevin [US/US]; 146 1st Street, Montara, CA 94037 (US).

(74) Agents: SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL THERAPEUTIC AGENTS THAT MODULATE ENZYMATIC PROCESSES

(57) Abstract

Novel multi-binding compounds are disclosed that modulate enzymatic processes. The compounds of the invention comprise from 2-10 ligands covalently connected, each of said ligands being capable of binding to an enzyme, enzyme substrate or enzyme cofactor thereby modulating the biological processes/functions thereof.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Novel Therapeutic Agents that Modulate Enzymatic Processes

Cross Reference to Related Applications

This application claims the benefit of United States Provisional Application Serial Numbers 60/088,448, filed June 8, 1998, and 60/093,072, filed July 16, 1998, both of which are incorporated by reference in their entirety.

Field of the Invention

This invention relates to modulators of enzymatic processes. More particularly, the invention relates to compounds that modulate enzymatic processes by acting as multibinding agents. The multibinding agents of the invention comprise from 2-10 ligands covalently connected by a linker or linkers, wherein said ligands in their monovalent (i.e. unlinked) state have the ability to bind to an enzyme, enzyme substrate, or enzyme cofactor, and are accordingly capable of modulating an enzymatic process. The manner in which the ligands are linked is such that the multibinding agents so constructed demonstrate an increased biological and/or therapeutic effect related to enzymatic processes as compared to the same number of unlinked ligands available for binding to the enzyme, enzyme substrate, or enzyme cofactor.

The compounds of the invention are useful for modulating enzymatic processes. They are in particular useful for treating pathologic conditions mediated in one form or another by enzymatic processes. Accordingly, the invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of the invention, and to methods of using such compounds and pharmaceutical compositions containing them for the treatment of such pathologic conditions.

Still further, the invention also relates to methods of preparing such compounds.

Background

Enzymes are the catalysts that mediate the chemical reactions of biological systems (for example, see: Walsh, C.T. 1979. *Enzymatic Reaction Mechanisms*. W.H. Freeman: New York; Fersht, A. 1985. *Enzyme Structure and Mechanism*. W.H. Freeman: New York; Boyer, P., ed.

1970-1976. *The Enzymes*, 3rd ed. 13 vols. New York: Academic Press). Through the processes of mutation and natural selection, enzymes have evolved into very efficient catalysts, and typically effect reaction rate accelerations of many orders of magnitude relative to uncatalyzed processes, and display high selectivity for catalysis of desired reactions relative to other processes.

It is clear that enzymes are critical to the survival of living organisms. However, because enzymes control the rate of biochemical reactions in metabolic pathways, they also have the potential for involvement in the development and persistence of pathological conditions. For example, autoimmune disorders (such as psoriasis, multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes, and the like) are characterized by excessive enzymatic production of inflammatory cytokines. Breast cancer and prostate cancer hormones at early stages of their development are dependent upon the enzymatic production of steroids. Blood clotting is regulated by the action of enzymes such as thrombin or Factor Xa, and thus undesired blood clotting activity can be modified by inhibiting the action of such enzymes. Hypertension can be treated by inhibiting angiotensin converting enzyme, and inflammation may be regulated by inhibition of cyclooxygenase production of inflammatory lipids. The treatment of neurodegenerative disorders, such as Parkinson's Disease, may be aided by treating patients with inhibitors of catechol *O*-methyl transferase (COMT). Inhibition of the activity of this enzyme improves the efficacy of treatment with the drug L-dopa and decreases the formation of 3-OMD, a metabolite of L-dopa with toxic effects.

Other examples of diseases in which enzymes are involved include infectious diseases caused by bacteria, protozoa or fungi, viral diseases such as AIDS (e.g., reverse transcriptase or protease enzymes), arthritis, metastatic cancer and tumor growth related to metalloprotease enzymes, stress and hypertension related to 11β-hydroxysteroid dehydrogenase and/or angiotensin.

Given the role played by enzymes in biological systems, it can be seen that enzymatic processes are probably involved at some level in all disease states or pathological conditions. Accordingly, the inhibition of enzymatic processes is regarded as an important target for drug development.

Any substance that reduces the rate of an enzymatic conversion of substrate to product is defined as an enzyme inhibitor. There are several fundamental mechanisms by which enzymatic

processes may be inhibited, for example reversible competitive inhibition, noncompetitive and uncompetitive inhibition, irreversible inhibition, substrate adulteration, and substrate sequestration. In reversible competitive inhibition, the inhibitor combines reversibly with free enzyme in a manner that excludes or reduces binding by the normal substrate for the enzyme. When a competitive inhibitor only reduces but does not totally exclude substrate binding, the inhibition is called partial competitive inhibition. In reversible noncompetitive inhibition, the inhibitor and substrate bind reversibly, randomly and independently at different sites. The enzyme:substrate:inhibitor complex is totally inactive or the rate of conversion of substrate to product is reduced in partial noncompetitive inhibition. In reversible uncompetitive inhibition, the inhibitor can only bind to the enzyme-substrate complex. Enzymes may also be inhibited irreversibly; e.g., they may undergo inactivating covalent modification by inhibitors. Irreversible inhibitors fall into two broad categories, depending upon whether they require pre-activation by the enzyme. Irreversible inhibitors such as reactive affinity labels, often used to probe enzyme active site structure, are intrinsically reactive with their target active site and require no pre-activation. In contrast, mechanism-based inactivators ("suicide substrates") are not intrinsically reactive with chemical functional groups on the enzyme, but these molecules are converted to reactive species in a process catalyzed at enzyme active sites. Finally, enzymes may be inhibited through mechanisms that do not involve direct interaction of the inhibitor with the enzyme. For example, inhibitors may bind to and sequester the substrate(s) for a given enzymatic process. In another possibility, inhibitors are activated by one enzyme and the activated species might inactivate or reversibly inhibit another enzyme, e.g. is isoniazid. Additionally, viral reverse transcriptase incorporates nucleotide analogs into growing DNA strands, which terminates the possibility for chain extension, thus inhibiting the subsequent enzymatic process ("substrate adulteration").

It has been estimated that approximately one-third of the targets of current commercial drugs are enzymes or enzymatic processes (Drews, J.; Ryser, S. *Nature Biotechnology* **1997).** Nonetheless, existing drugs have many disadvantages, including lack of selectivity for the targeted enzyme, low potency, short duration of action, toxicity, and the like.

Accordingly, it would be advantageous to discover enzyme-inhibiting compounds that demonstrate high activity and selectivity toward target enzymes, and are of low toxicity. It would also be desirable to find a method for designing such compounds.

Summary of the Invention

This invention addresses the above needs by providing novel multibinding agents. Accordingly, in one aspect, the present invention relates to novel multibinding agents

wherein a multibinding agent comprises 2-10 ligands, which may be the same or different, covalently connected by a linker or linkers, which may be the same or different, each of said ligands compromising a ligand domain capable of binding to an enzyme, enzyme substrate, or enzyme cofactor..

The preferred multibinding agents are represented by Formula I:

$$(L)_p(X)_q$$

Formula I

in which L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of 2-10; and

q is an integer of 1-20;

or a salt thereof;

wherein each of said ligands comprises a ligand domain capable of binding to an enzyme, enzyme substrate, or enzyme cofactor, thereby modulating the activity of the enzyme, enzyme substrate, or enzyme cofactor. Preferably q is less than p.

In a second aspect, the invention relates to a method of inhibiting enzymatic processes in a biologic tissue, preferably in a mammalian or avian subject, comprising administering to a subject in need of such treatment a therapeutically effective amount of a multibinding agent; wherein a multibinding agent comprises 2-10 ligands, which may be the same or different, covalently connected by a linker or linkers, which may be the same or different, each of said ligands compromising a ligand domain capable of binding to an enzyme, enzyme substrate, or enzyme cofactor.

In a third aspect, the invention relates to a method of inhibiting enzymatic processes in a biologic tissue, preferably in a mammalian or avian subject, comprising administering to a subject in need of such treatment a therapeutically effective amount of a compound of Formula I, or a salt thereof.

In a fourth aspect, the invention relates to a pharmaceutical composition comprising a therapeutically effective amount of one or more multibinding agents, or a pharmaceutically

acceptable salt thereof, said multibinding agent comprising 2-10 ligands, which may be the same or different, covalently connected by a linker or linkers, which may be the same or different, each of said ligands compromising a ligand domain capable of binding to an enzyme, enzyme substrate, or enzyme cofactor, admixed with at least one pharmaceutically acceptable excipient.

In a fifth aspect, the invention relates to a pharmaceutical composition comprising a therapeutically effective amount of one or more compounds of Formula I, or a pharmaceutically acceptable salt thereof, admixed with at least one pharmaceutically acceptable excipient.

In a sixth aspect, the invention relates to a method for identifying a multibinding agent capable of binding to an enzyme, comprising preparing an array of multimeric agents, contacting the multimeric agent array with an enzyme, enzyme substrate, or enzyme cofactor, and slecting a multibinding agent based upon its ability to bind to the enzyme.

In a sixth aspect, the invention relates to a method for identifying a multimeric ligand compound that possesses multibinding properties with respect to enzymes, comprising preparing an array of multimeric agents, contacting the multimeric agent array with an enzyme, and selecting a multibinding agent based upon its ability to bind to that enzyme.

In a seventh aspect, the invention relates to processes for preparing the compounds of Formula I.

BRIEF DESCRIPTION OF THE DRAWINGS

Reaction Schemes 1-16 and the Figures and Tables of the Appendix illustrate various aspects of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Biological systems in general involve molecular interactions between bioactive ligands and their receptors, in which the receptor "recognizes" a molecule (which may be a ligand or a substrate) or portion of a molecule (or ligand domain). Thus, enzymatic processes involve interaction of an enzyme with a ligand or substrate that is "recognized" by the enzyme. The result of this interaction can be either to initiate the desired biological effect of the enzyme, or alternatively to inhibit or alter (i.e. to modulate) the normal function of the enzyme. As noted above, whereas enzymes are critical to the survival of living organisms, they also play a role in disease states, and the inhibition of enzymatic processes is regarded as an important target for drug development.

The interaction of an enzyme with a ligand may be described in terms of "affinity" and "specificity". The affinity and specificity of any given ligand/enzyme interaction are dependent upon the complementarity of molecular binding surfaces and the energetic costs of complexation. Affinity may be quantified by the equilibrium constant of complex formation. Specificity relates to the difference in affinity between different ligands binding to the same receptor (here an enzyme, enzyme substrate, or enzyme cofactor), or the same ligand binding to different receptors. The compounds of the invention are multibinding agents, and although not wishing to be bound or restricted by any particular theory or proposed mechanism of action, it is believed that the surprising activity of these compounds at least in part arises from their ability to bind in a multivalent manner with the enzyme receptor, enzyme substrate or enzyme cofactor, and thus lower the energetic costs of binding to the enzyme, enzyme substrate, or enzyme cofactor. Multivalent binding interactions are characterized by the concurrent interaction of at least two ligands of a multibinding agent with multiple ligand binding sites (enzymes, enzyme substrate, or enzyme cogactors), which may be multiple distinct enzymes or multiple distinct binding sites on a single enzyme. Multivalent interactions differ from collections of individual monovalent interactions in that they give rise to an enhanced biological effect.

Definitions

As used herein:

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 carbon atoms, such as methyl, ethyl, n-propyl, is opropyl, n-butyl, secondary butyl, tert-butyl, n-hexyl, n-octyl, n-decyl, n-dodecyl, 2-ethyldodecyl, tetradecyl, and the like, unless otherwise indicated.

The term "substituted alkyl" refers to an alkyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-aryl, -

SO_2-heteroaryl, and -NR^aR^b, wherein R^a and R^b may be the same or different and and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 carbon atoms. This term is exemplified by groups such as methylene (-CH_2-), ethylene (-CH_2CH_2-), the propylene isomers (e.g., -$CH_2CH_2CH_2$- and -$CH(CH_3)CH_2$-) and the like.

The term "substituted alkylene" refers to:

(a) an alkylene group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino (including, for example, N-glucosaminecarbonyl, benzoylamino, biphenylcarbonylamino, and the like), acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocyclooxy, nitro, and -NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group.

(b) an alkylene group as defined above that is interrupted by 1-20 atoms or substituents independently chosen from oxygen, sulfur and NR^a-, wherein R^a is chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic; or

(c) an alkylene group as defined above that has both from 1 to 5 substituents as defined above and is also interrupted by 1-20 atoms as defined above.

Examples of substituted alkylenes are chloromethylene (-CH(Cl)-), aminoethylene (-$CH(NH_2)CH_2$-), 1-(dodecanoylamino)propylene (-CH[NHC(O)-$(CH_2)_{11}$-CH_3] CH_2-), 1-(4-phenylbenzoylamino)pentylene (-CH[NHC(O)-Z] $(CH_2)_4$) ,2-carboxypropylene isomers (-$CH_2CH(CO_2H)CH_2$-), ethoxyethyl (-CH_2CH_2 O-CH_2CH_2-), -), ethylmethylaminoethyl (-CH_2CH_2

$N(CH_3)$ CH_2CH_2-), 1-ethoxy-2-(2-ethoxy-ethoxy)ethane (-CH_2CH_2 O-CH_2CH_2-O-CH_2CH_2 O-CH_2CH_2-), and the like.

The term "alkaryl" or "aralkyl"refers to the groups -alkylene-aryl and -substituted alkylene-aryl in which alkylene and aryl are as defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, n-propoxy, is o-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Examples of such groups are methylenemethoxy (-CH_2OCH_3), ethylenemethoxy (-$CH_2CH_2OCH_3$), n-propylene-iso-propoxy (-$CH_2CH_2CH_2OCH(CH_3)_2$), methylene-t-butoxy (-CH_2-O-$C(CH_3)_3$) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy (-CH_2SCH_3), ethylenethiomethoxy (-$CH_2CH_2SCH_3$), n-propylene-iso-thiopropoxy (-$CH_2CH_2CH_2SCH(CH_3)_2$), methylene-t-thiobutoxy (-$CH_2SC(CH_3)_3$) and the like.

"Alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as vinyl, prop-2-enyl, pent-3-enyl, hex-5-enyl, 5-ethyldodec-3,6-dienyl, and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1

to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, aryloxy, thioaryloxy, heteroaryloxy, thioheteroaryloxy, heterocyclooxy, thioheterocyclooxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, and. -NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkenylene" refers to a diradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as 1,2-ethenyl, 1,3-prop-2-enyl, 1,5-pent-3-enyl, 1,4-hex-5-enyl, 5-ethyl-1,12-dodec-3,6-dienyl, and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocyclooxy, nitro, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

"Alkynyl" refers to a monoradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as acetylenyl, prop-2-ynyl, pent-3-ynyl, hex-5-ynyl, 5-ethyldodec-3,6-diynyl, and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen,

hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocycloxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, SO_2-heterocyclic, NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkynylene" refers to a diradical of an unsaturated hydrocarbon radical, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as 1,3-prop-2-ynyl, 1,5-pent-3-ynyl, 1,4-hex-5-ynyl, 5-ethyl-1,12-dodec-3,6-diynyl, and the like.

The term "acyl" refers to the groups -CHO, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acylamino" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g.,

naphthyl or anthryl).

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, trihalomethyl, NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to a diradical derived from aryl or substituted aryl as defined above, and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "carboxyalkyl" refers to the group "-C(O)Oalkyl" where alkyl is as defined above.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -

SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring or fused rings and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

"Haloalkyl" refers to alkyl as defined above substituted by 1-4 halo groups as defined above, which may be the same or different, such as 3-fluorododecyl, 12,12,12-trifluorododecyl, 2-bromooctyl, -3-bromo-6-chloroheptyl, and the like.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy,

thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, trihalomethyl, mono-and di-alkylamino, mono- and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred heteroaryls nclude pyridyl, pyrrolyl and furyl.

The term "heteroaryloxy" refers to the group heteroaryl-O-.

The term "heteroarylene" refers to the diradical group derived from heteroaryl or substituted heteroaryl as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylene, 1,8-quinolinylene, 1,4-benzofuranylene, 2,5-pyridinylene, 1,3-morpholinylene, 2,5-indolenyl, and the like.

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Such heterocyclic groups can have a single ring or multiple condensed rings.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, is oindole, indole, indazole, purine, quinolizine, is oquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, is othiazole, phenazine, is oxazole, phenoxazine, phenothiazine, imidazolidine,

imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

A preferred class of heterocyclics include "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula $[-(CH_2-)_mY-]$ where m is equal to or greater than 2, and Y at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only, $[-(CH_2)_3-NH-]_3$, $[-((CH_2)_2-O)_4-((CH_2)_2-NH)_2]$ and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group derived from a heterocycle as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

"Alkyl optionally interrupted by 1-5 atoms chosen from O, S, or N" refers to alkyl as defined above in which the carbon chain is interrupted by O, S, or N. Within the scope are ethers, sulfides, and amines, for example 1-methoxydecyl, 1-pentyloxynonane, 1-(2-isopropoxyethoxy)-4-methylnonane, 1-(2-ethoxyethoxy)dodecyl, 2-(t-butoxy)heptyl, 1-pentylsulfanylnonane, nonylpentylamine, and the like.

"Heteroarylalkyl" refers to heteroaryl as defined above linked to alkyl as defined above, for example pyrid-2-ylmethyl, 8-quinolinylpropyl, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, optionally substituted alkyl means that alkyl may or may not be substituted by those groups enulerated in the definition of substituted alkyl.

"Ligand" as used herein denotes a compound that is a binding partner for an enzyme or an enzyme substrate and is bound thereto by complementarity. The specific region or regions of the ligand that is (are) recognized by the enzyme is designated as the "ligand domain". By virtue of the ligand domain, a ligand may be either capable of binding to an enzyme, enzyme substrate, or enzyme cofactor by itself, or may require the presence of one or more non-ligand components for binding (e.g. Ca^{2+}, Mg^{2+}, or a water molecule is required for the binding of a ligand domain to various receptors).

Examples of ligands useful in this invention are given below and in the Appendix (Table 1).

Enzyme/ Enzymatic Process	Current and Potential Therapeutic Indication(s)	Inhibitory Drugs and Other Ligands
Oxidoreductase Enzymes		
Aldose reductase (1.1.1.21)	Diabetic retinopathy Diabetic neuropathy Diabetic complications Cataracts Diabetic foot ulcer Diabetic nephropathy	CT-112, zenarestat, minalrestat, AD-5467, zopolrestat, SX-3201, NZ-314, ZD-5522, imirestat, sorbinil, M-16209, GP-1447, tolrestat, M-16049, FR-62765, DN-108, SC-103a, AND-138, AL-1567, SNK-860, thiazocin-A, SPR-210, E-0722, AL-4114, WP-921, epalrestat, WF-2421, BAL AR-18
HMG-CoA reductase (1.1.1.34)	Hypercholesteremia Hyperlipidemia Atherosclerosis	Mevastatin, lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, dalvastatin, nisvastatin, BB-476, L-659699, glenvastatin, CP-83101, BMS-180431, SR-12813, DMP-565, L-669262, carvastatin, S-2467, S-2468, PD-135022, SQ-33600, crilvastatin, rawsonol, bervastatin, S-4522, acitemate, U-9888, U-20685, U-51862, U-71690
Inosine 5'-phosphate dehydrogenase (1.1.1.205)	Viral infection Cancer Transplant rejection Kidney disease Autoimmune disease Psoriasis Rheumatoid arthritis Depression	Ribavirin, tiazofurin/TAD, β-methylene-BAD, mizoribine, CI-935, thiophenfurin, puberulronic acid, VX-497
Steroid 5-alpha reductase 1 and 2 (1.3.99.5)	Prostatic hypertrophy Prostate tumor Male-pattern baldness Acne	Finasteride, epristeride, LY191704, FK-143, MK-906, MK-0434, turosteride, 4-MA, GW-747, L-654066, ONO-3805, PNU-157706, LY-266111, d1067331, L-751788, MK-386, Z-350

Dihydroorotate dehydrogenase (1.3.99.11)	Carcinoma Immune disorder Neoplasm Organ transplantation Psoriasis Rheumatoid arthritis Multiple sclerosis	NSC-339768, brequinar, leflunomide (launched), DuP-785, KF-20444, MNA-715
Monoamine oxidase A and B (1.4.3.6)	Migraine Depression Anxiety Parkinson's disease Alzheimer's disease Neurodegenerative disease Dementia	Phenelzine, isocarboxazid tranylcypromine, selegiline, mofegiline, esuprone, RS-8359, KP-157, IFO, E-2011, lazabemide, befloxatone, T-794, parafluoroselegiline, bifemelane, RO-41-1049, P-11345, P-11467, rasagiline, 1370U, L-650477, indeloxazine, moclobemide, brofaromine, EXP-631
Dihydrofolate reductase (1.5.1.3)	Cancer Psoriasis Inflammation Rheumatoid arthritis Asthma Neoplasm Leukemia Hematologic disease Lymphatic system disease	Methotrexate, edatrexate, piritrexim, LY-298207, LY-316373, 1954U89, E-34335, AG-384, AG-350, TNP-351, MDAM, LY-335518, AG-394, LY-335580, LY-335738, LY-28874
Lipid peroxidase (1.11.1.3)	Alzheimer's disease Cognitive disorder Neurodegenerative disease	GYKI-24417, idebenone (launched)
5-Lipoxygenase (1.13.11.34)	Inflammation Asthma Rheumatoid arthritis Osteoarthritis Irritable bowel syndrome Ulcerative colitis Dermatitis Pruritis	Zileuton, docebenone, ICI-D2318, MK-0591, MK-886, piripost, tenidap, AA-861, PF-5901, L-656224, ABT-761, Bay-x-1005, ZD-2138, WY-28342, tenidap, CI-1004, BW-B70C, A-79175, CGS-25997, BI-L-357, SKF-105809, ZD-4407, 3323W, tepoxalin, T-0799, CGS-26529, A-63162, SKF-104351, SC-45662, CMI-977, A-81834, FR-110302, P-10294, BW-A4C, E-6700, ICI-211965, CMI-568, L-697198, PGV-20229, L-7080780, MK-866, BW-137C, A-72694, L-739010, WY-50295-tromethamine, SC-41661A, P-8977, LY-233569, L-670630, CI-986, tagorizine, FR-122788, ER-34122, L-705302, PD-138387, BF-389, SKF-107649, ZM-216800, CMI-206, chrysarobin analogs, ML-3000, FPL-64170, CGS-23885, ONO-LP-049, L-702539, flobufen, ETH-615, SB-210661, A-121798, YPE-01, HX-0836, E-6080, SKF-86002, tebufelone, Sch-40120, LY-221068, lonapalene, DuP-983, CBS-113-A, T-0757, CMI-392, KC-11425, BTS-71321, KC-11404, ZD-2138, WAY-125007, linazolast, E-3040, ABT-080, PD-089244, A-93178, RWJ-63556, nicaraven
Nitric Oxide Synthase (1.14.13.39)	Hypotension Septic shock Inflammation Vascular disease Rheumatoid arthritis Cerebrovascular ischemia Head trauma Neuropathy Carcinoma Neurodegenerative disease	L-NMMA, HMN-1180, A-84643, 3936W92, 546C88, HF-2035, SC-59039, CHU-148, AR-R-17477, BN-80933, 7-nitroindazole, ARL-16566
Sterol 14-alpha demethylase (1.14.14.1)	Fungal infection Parasitic infection Atherosclerosis Hypercholesterolemia Hyperlipidemia Neoplasm	Ketoconazole, fluconazole, itraconazole, clotriamzole, miconazole, UR-9825, UR-9746, UR-9751, Sch-56592, T-8581, Sch-42538, Sch-42427, GR-99060, Sch-45012, UR-9728, Sch-51048, UR-9717, ZD-0870, voriconazole, BMS-207147, Sch-50002, becliconazole, A-39806, azalanstat, etanidazole

Cyclooxygenase 1 (1.14.99.1)	Inflammation Psoriasis Pruritis Thromboembolism Cerebrovascular ischemia Artery disease Rheumatoid arthritis Pain Edema Carcinoma Leukemia Migraine Glaucoma Ocular disease Transplant rejection Neoplasm Multiple sclerosis Tumor Musculoskeletal disease Platelet aggregation	Aspirin, ibuprofen, flurbiprofen, indomethacin, acetaminophen, tolmetin, mefanmic acid, CI-1004, P-10294, P-8977, pamicogrel, WY-28342, nitroflurbiprofen, NCX-4016, lornoxicam, ML-3000, tenidap, CS-670, FS-205-397, eltenac, CI-986, N-14, SKF-86002, SKF-105809, RP-66364, pirazolac, P-8892, ER-34122, tepoxalin, flobufen, HN-3392, CBS-113-A, BF-389, SC-57949, PD-145246, E-5110, FR-122047, tenoxicam, LCB-1892, PD-136005, dexibuprofen, BW-755-C, HCT-2035, FR-140423, nabumetone, ketorolac, aceclofenac, naprelan, exisulind, mepranoprofen, D-1367, CGP-31081, etodolac, pemedolac, paracetamol, WAY-120739, triflusal, KC-764, S-14080, felbinac, droxicam, bromfenac, piroxicam, bermoprofen, amtolmetin, ampiroxicam, tenosal, trifenagrel, HX-0836
Cyclooxygenase 2 (1.14.99.1)	Inflammation Psoriasis Pruritis Thromboembolism Cerebrovascular ischemia Artery disease Rheumatoid arthritis Osteoarthritis Colon tumor Pain Alzheimer's disease Edema Carcinoma Leukemia Migraine Glaucoma Ocular disease Transplant rejection Neoplasm Multiple sclerosis Musculoskeletal disease Platelet aggregation	Meloxicam, L-745337, pamicogrel, P-9877, P-10294, WY-28342, nitroflurbiprofen, PGV-20229, NCX-4016, celecoxib, lornoxicam, SC-57666, ML-3000, tenidap, CS-670, SC-58451, L-768277, L-783003, GR-253035, FS-205-397, eltenac, CI-986, FR-123826, N-14, PD-164387, SKF-86002, SKF-105809, RP-66364, pirazolac, P8892, nimesulide, rofecoxib, L-761066, ER-34122, L-791456, PD-138387, tepoxalin, flobufen, HN-3392, CBS-113-A, BF-389, SC-57949, PD-145246, NS-398, E-5110, FR-122047, tenoxicam, LCB-1892, PD-136005, dexibuprofen, BW-755-C, JTE-522, RWJ-63556, HCT-2035, SC-58231, L-746483, XU-745, L-748731, FR-140423, L-748780, CS-179, BIRL-790,, A-183827.0, nabumetone, ketorolac, aceclofenac, naprelan, leflunomide, mepranoprofen, D-1367, SC-58125, L-776967, vioxx, SC-59046, SC-236, L-784512, SC-65872, flosulide, CGP-31081, etodolac, pemedolac, paracetamol, WAY-120739, triflusal, KC-764, S-14080, felbinac, DuP-697, droxicam, bromfenac, piroxicam, bermoprofen, amtolmetin, ampiroxicam, SC-69124A, DFP, M-5011, L-758115, tenosal, DFU, trifenagrel, CT-3, RS-57067-000, PD-098120-0003, L-752860, HX-0836, diclofenac, SC-58236
Squalene monooxygenase (1.14.99.7)	Fungal infection Atherosclerosis Hypercholesterolemia Hyperlipidemia Coronary artery disease	Terbinafine, naftifine, NB-598, FR-194738, SDZ-87-469, Ro-44-2104, FW-1045, SDZ-880-540
Steroid 17-alpha hydroxylase (1.14.99.9)	Prostate tumor Atherosclerosis Hypercorticism Cushing's syndrome	Lifibrol (activator) Nilutamide (launched), vorozole abiraterone, L-36, CB-7661, CB-7645, 3- and 4-pyridyl adamantanecarboxylates, YM-116, GI-111924, CB-7661, YM-55208, liarozole, metyrapone
Transferase Enzymes		
Thymidylate synthase (2.1.1.45)	Breast tumor Carcinoma Colorectal tumor Lung tumor Pancreas tumor Head & neck tumor Liver tumor Prostate tumor Solid tumor Uterine cervix tumor Bladder tumor Urinary tract tumor Renal tumor	Raltitrexed, LY-231514, lipophilic quinazoline derivatives, GW-1843, 5-arylthio-substituted 2-amino-4-oxo-6-methylpyrrolo[2,3-d]pyrimidine, AG-337, ZD-9331, ICI-198583, ZD-1694, ZM-246315, FO-152, LY-225693, gemcitabine (launched), CB-30900, DMPDDF, AG-331, trimetrexate, CB-300638, CB-300907, piritrexim, doxifluridine (launched), galocitabine

Ribosomal protein biosynthesis (50S ribosomal subunit) (2.3.2.12)	Bacterial infection Pneumocystis carinii infection	Chloramphenicol, erythromicin, clarithromycin, azithromycin, dirithromycin, flurithromycin clindamycin, lincomycin, quinopristin, dalfopristin, streptogramins, linezolid, U-100480, U-101603, U-94901, U-101244, pristinamycin, MJ-347-81F4A, HMR-3647, L-708299, A-184656, L-708365, L-701677, lexithromycin, RU-64004, CP-227182, CP-426027, TEA-0769, CP-279107, RU-56006, RU-6652, RU-59616, L-744434, L-744433, L-740893, L-709936, leucomycin, A-179796, eperezolid, U-100480
Ribosomal protein biosynthesis (Aminoacyl tRNA site on 30S ribosomal unit) (2.3.2.12)	Anti-bacterial	Tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, CL-331002, glycylcyclines, CL-331928, CL-344667, CL-329998, PAM-MINO
Ribosomal protein biosynthesis (30S subunit) (2.3.2.12)	Anti-bacterial	Streptomycin, gentamicin, tobramycin, amikacin, netilimicin, kanamycin, neomycin, spectinomycin, dactimicin, paromomycin, trospectomycin
Ribosomal protein biosynthesis (soluble protein factors) (2.3.2.12)	Anti-bacterial	Fusidic acid, purpuromycin
Glucan synthase (2.4.1.34)	Fungal infection Protozoal infection	LY-303366, pneumocandins, pneumocandin analogs, echinocandins, echinocandin analogs, MK-0991, LY-307853, FR-901379, L-705589, L-731373, L-733560, aureobasidins, aureobasidin analogs, cepacidine A, L-688786, L-671329, L-692289, L-733686, cilofungin, basifungin, deoxymulunocandin
Transglycosylase (2.4.1.129)		Vancomycin, teicoplanin, eremomycin, mideplanin, LY-333328, LY-191145, M-16459, decaplanin, BI-397, A-40926, MM-55266, MM-55268, chloroorienticin
Purine nucleoside phosphorylase (2.4.2.1)	Lymphoma Gout Transplant rejection Leukemia Immune disorder Dermatitis Psoriasis Non-Hodgkin's lymphoma Rheumatoid arthritis Multiple sclerosis Ocular disease HIV infection Autoimmune disease Carcinoma Inflammation Neoplasm Viral infection	Fluorinated purinyl phosphonic acids, BCX-5, peldesine, CI-950, 3,3-dimethylpentyl-9-substituted guanine analogs, CI-972, CH-377, CH-424, CH-799, MDL-74428, acyclovir analogs
Epidermal growth factor receptor tyrosine kinase (2.7.1.112)	Neoplasm Carcinoma Breast tumor Restenosis Inflammation Solid tumor Bladder tumor Angiogenesis disorder Head and neck tumor Non-Hodgkin's lymphoma Psoriasis	ZM-105180, PD-158780, SU-4984, SU-5402, SU-1498, CGP-52411, ZD-1839, PD-165557, PD-166075, CGP-59326, CP-358774, genistein, PD-090560, CEP-701, 4-(2-diethylaminoethoxy)-aminopyrido[2,3-d]pyrimidin-7(8H)-one, PD-171026, PD-151514, 7,8-dimethoxy-5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one, CGP-52411, CGP-53353, CGP-56214, DAB-720, RG-14620, PD-168393, PD-160678, PD-169414, BE-23372M, naamidine A, SU-5271, CGP-60621, ZD-1839, CGP-62706, CGP-59326, SU-65847, PD-169450, RG-13022, semicochliodinols, ZD-1938, ZD-1839, CGP-79787, ZM-105180, AR-639

Platelet-derived growth factor receptor tyrosine kinase (2.7.1.112)	Neoplasm Psoriasis Arteriosclerosis Carcinoma Inflammation Restenosis Cardiovascular disease Ovary tumor Brain tumor Solid tumor Prostate tumor Glomerulonephritis	PD-171026, SU-4984, SU-5402, SU-1498, phenylamino-pyrimidines, leflunomide, PD-089828, genistein, PD-090560, CEP-701, 4-(2-diethylaminoethoxy)-aminopyrido[2,3-d]pyrimidin-7(8H)-one, PD-171026, PD-151514, 7,8-dimethoxy-5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one, CGP-52411, SU-65847, RPR-1015119, CGP-79787, SU-65786, SU-102, B-1146 compounds, KI-6896, CGP-53716, leflunomide, 3-(4-Pyridinyl)quinolines, SU-6668
Basic fibroblast growth factor receptor tyrosine kinase (2.7.1.112)	Neoplasm Cardiovascular disease Prostate tumor	PD-089828, PD-090560, CEP-701, 4-(2-diethylaminoethoxy)-aminopyrido[2,3-d]pyrimidin-7(8H)-one, PD-171026, PD-151514, 7,8-dimethoxy-5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one, CGP-52411, RG-8803, BP-42, SU-6668
Beta subunit of DNA-dependant RNA polymerase (2.7.7.6)	Bacterial infection	Rifampin, rifabutin, rifalazil, T9, SPA-S-565
DNA polymerase DNA directed (2.7.7.7)	Digestive tract tumor Carcinoma Viral infection Pancreatic tumor Ovary tumor Prostate tumor Lung tumor Leukemia Solid tumor	KN-208, aphidicolin, KM-043, netivudine, A-79296, BMS-181165, E-EBU-dM, BMS-200475, MPC-531, L-732801, 773U82, FTC, delaviridine, U-9843, GR-95168, ganciclovir, ABT-606, FLG, robustaflavone, BHAP-E, amitivir, valaciclovir, famciclovir, (N-((5-ethyl-2-methoxy-6-methyl-3-pyridyl)methyl)amino)-5-ethyl-6-methylpyridin-2(1H)-one, lamivudine, zidovudine, phosphonoformate, atevirdine, cytarabine, netivudine, cyclobut-G, U-80493, miboplatin, cis-platin, carboplatin, JM-118, JM-126, fialuridine, tallimustine, distamycin A, netropsin, CC-1065, epelmycin A, aciclovir
Reverse transcriptase (2.7.7.49)	Viral infection HIV infection Carcinoma Neoplasm Cytomegalovirus infection Hepatitis virus infection Cancer	Zidovudine, didanosine, stavudine, zalcitabine, suramin, efavirenz, delaviridine, L-696229, BHAP-E, oltipraz, abacavir, lodenosine, L-697639, nevirapine, E-EBU-dM, troviridine, BM-51.0836, MEN-10690, adefovir, raluridine, loviride, UC-38, talviraline, SKI-1695, mniopetals, L-697661, L-737126, R-18893, R-82150, PMPA, CS-92, UC-84, BM-21.1298, MKC-442, lentinan, azidouridine, Ro-25-0236, MSC-127, L-732801, FTC, U-9843, IVX-E-59, lamivudine, tivirapine, calanolide A/B, L-738372, MSA-300, isoddA, DABOs, LY-326594, suramin, inophyllum B, PMTI, PMTG, RD4-2217, imidine, thiadiazines, benzimidazoles, S-DABO compounds, KM-043, BCH-10619, PNU-142721, MSH-372, azido-ddG, ADRT, A-79296, EA-521, NSC-645542, S-1153, beta-L-Fd4C, ateviridine, SJ-3366, michellamine B, TSAO-T, PC-1250, WIN-49611, MSC-168, SKI-1703, L-608788, MEN-10880, MEN-10979, MPC-531, JM-2820, JM-1596, JM-1591, L-734005, TGG-II-23A, S-2720, MSF-294, MSH-143, L-297345, LY-73497, U-90328, cyclobut-G, didanosine, BRL-47923-DP, DADP, NSC-625487, RD4-2025, NSC-667952, UC-781, FLG, MSC-204, BEA-005, U-31355, sulfoxamine, CGP-53437, sorivudine, G-3139, ganciclovir, acyclovir, KP-21, amitivir, d4T derivatives, ddI, ribavirin, BCH-10618, BCH-4989, Hoe/Bay-946, R-82913, L-229, valaciclovir, U-80493, BSU-1051, PASs, AZT-P-ddI, lexithromycin, HEPT analogs, fialuridine, famciclovir, C-AFG, BCH-10652, Dioxolane-T, A-5021, SPV-30, VF-1618, TNK-651

D,D-transpeptidases (PBP 2a; 2b) **D,D-transpeptidases (PBP 1a)** **D,D-Carboxypeptidase (PBP 3-7) (3.4.16.4)**	Bacterial infection	Beta-lactams Penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, carbenicillin, carbenicillin indanyl, ticarcillin, mezlocillin, piperacillin, cephalothin, cefazolin, cephalexin, cefadroxil, cefamandole, cefoxitin, cefaclor, cefuroxime, loracarbef,cefonicid, cefotetan, ceforanide, cefotaxime, cefpodoxime proxetil, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefepime, imipenem, meropenem, aztreonam, ritipenem, L-695256, GV-143253, sanfitrinem, fropenem, lactivicin, BO-2727, MEN-10700, Ro-48-8724, cefosilis, SB-216477, S-4661, GG-326, BLA-857, PGE-8335534, PGE-542860, LB-10522, GV-129606, BO-2052A, CS-834, MK-826, YH-1226, YM-40220, MDL-63908, FCE-25199, panipenem, TOC-50, TOC-39, TOC-29, E-1101, sulopenem, DU-6681, MC-02479, temocillin, carumonam, Ro-25-0534, SUN-A-0026, WS-1358A, Ro-25-1132, CGP-57701, CGP-37697A, TMA-230, Syn-2190, biapenem, CS-834, DWP-204, DX-8739, CS-976, CKD-529, ER-35786, DZ-2640, 4-AAZ, KR-21012, Ro-25-0993, DA-1211, BMS-181139, J-111225, L-786392, DK-35C, Ro-25-6833, S-1090, E-1101, FK-518, KP-736, cefditoren, LY-215891, Ro-09-1428, cefdaloxime, cefoselis, KST-150185, Ro-09-1227, cefclidin, cefluprenam, cefotiam, LB-10522, cefcanel, BRL-57342, cefpirome, YH-1226, cefprozil, CKD-604, KST-150288, cefcapene, Ro-24-8138, FK-312, cefozopran, RU-59863, ceftibuten, FR-193879, FK-041, cefdinir, CP-6679, Ro-63-9141, CFC-240, cefpimizole, cefminox, cefetamet, CP-0467, PGE-7119699, Ro-48-8391, AM-1817, AM-1732, MC-02002, BO-1341, BK-218, Ro-25-4835, Ro-25-2016, YM-40220, Ro-23-9424, LY-206763, CR-240, YH-1266, MC-02331, Ro-44-3949, MC-02306, Ro-25-7103, BMS-180680
Hydrolase Enzymes		
Type V cyclic GMP phosphodiesterase (3.1.4.35)	Platelet aggregation Hypertension	Zaprinast, dipyridamole, sildenafil, WIN-58237, UK-114542, NM-702, SKF-96231, IC-351, WIN-65579, EMD-82639, Sch-51866, UK-83405, WS-63967, M-54033, FK-336, GP-385, HMN-709B, 5E-3623, 5E-3569, 5E-3657
Type IV cyclic AMP Phosphodiesterase (3.1.4.17)	CNS modulation Gastric acid secretion Asthma Inflammation Rheumatoid arthritis COPD Cardiac failure	Rolipram, RO-020-1724, atizoram, piclamilast, WAY-127093B, filaminas, CDP-840, GW-3600, YM-976, DWP-205 derivatives, compound 27, RS-25344-000, SB-207449, NA-23063, CH-3697, T-440, CH-673, CH-422, D-22888, CH-928, CP-293321, V-11294A, RPR-114597, SKF-107806, tolafentrine, pentoxifylline, cipamfylline, NCS-613, A-906119, E-4021, Org-30029, Org-20241, losartan derivatives, WAY-126120, RPR-132294, LAS-30989, LAS-31396, D-4418, arolfylline, CP-220629, AWD-12-281, RP-73401, isomazole, D-4418
Neuraminidase (3.2.1.18)		Zanamivir, GS4104
Angiotensin-converting enzyme (3.4.15.1)	Hypertension Left ventricular systolic dysfunction Myocardial infarction Scleroderma renal crisis Heart failure Diabetic neuropathy Pain Opiate use disorder Cerebrovascular ischemia	Captopril, fentiapril, pivalopril, zofenopril, alacepril, enalapril, lisinopril, benazepril, quinapril, moexipril, ramipril, spirapril, perindopril, indolapril, pentopril, indalapril, cilazapril, fosinopril, CGS-30440, ceronapril, zabiciprilat, RB-106, temocapril, trandolapril, mixanpril, MDL-102769, benzofused macrocyclic lactams, sampatrilat, UK-79942, UK-63831, CGS-28106, BMS-182657, MDL-100240, AB-47, moveltipril, imidapril, RB-105, ER-32897, ER-32935, CGS-27025, DU-1777, Z-13752A, Sch-54470, ER-32945, BMS-189921, MDL-27088, BRL-36378, libenzapril, utibapril, synecor, quinaprilat, RB-101, RB-120, FPL-66564, delapril, moexiprilat, SC-50560, prentyl, MDL-27467A, RL-6134, idrapril, cyclic diazepinones, fasidotril, GI-155704A, zofenopril, CGS-26670, SA-7060, omapatrilat

Thrombin (3.4.21.5)	Anti-coagulation Angina Blood clotting disorder Cerebrovascular disease Deep vein thrombosis Myocaridal infarction Thrombocytopenia Thromboembolism Lung embolism Pancreatitis	Heparin, low molecular weight heparin, DHG, argatroban, desirudin, bivalirudin, CVS-1123, BCH-1710, DuP-714, inogatran, CVS-995, LY-293435, theromin, TRI-50b, LY-303496, SR-80027A, RWJ-50353, melagatran, L-371912, bufrudin, TRI-166, LR-D/009, SD-523, BCH-2763, UK-156406, L-372460, diarylsulfonamides, L-373890, UK-239326, L-373363, L-375052, L-636619, CVS-1801, GR-133487, APC-104, TRIB50, BMS-189664, RWJ-50305, SR-32701, L-375378, GS-522, LY-298952, C-186-65, LEX-026, SDZ-MTH-958, BMS-180053A, MOL-144, S-18326, L374087, S-17436, napsagatran, BMS-182627, MDL-75579, MDL-73775, MDL-73446, LY-806303, BMY-43392-A, efegatran, CRC-305, CRC-220, RWJ-27755, Ro-09-1679, MDL-73756, BMY-44621, FR-171113, vasoflux, 4H12, triabin, hirunorm, 3DP-4147, MOL-126, S-30580, LU-57291, CX-397, LY-329186, CVS-1578, CVS-1897, P-551, P-532, Org-37432, Org-34092, RWJ-51438, TMD1-105, SDZ-217-766, lepirudin, ardeparin, thrombostatins, MOL-097, TTS24, H-376/95, GR-133686, CVS-2038, CVS-2287, LY-333545, LY-355429, TRI-74, Org-36764, LB-30057, CVS-1862, CVS-1304, CVS-863, 3DP-4026, L-372236, BMS-189090, CVS-1578, MOL-098, MOL-127
Factor Xa (3.4.21.6)	Thrombosis Deep vein thrombosis Disseminated intravascular coagulation Myocardial infarction Angina Lung embolism Thromboembolism Cerebrovascular ischemia	NAP-5, NAP-A, DX-9065A, arylsulfonamidopiperazine, 2,3-disubstituted beta-alanines, (tetrahydroisoquinolyloxy)phenylacetic acid derivatives, ZK-805350, BX-807834, ZK-807191, C-92178, 2,4-diazepin-3-one derivatives, rTAP, Cordecin AS, SK-549, DHG, FX-2212, SEL-2711, yagin, BM-141248, ZK-806350, ZK-807191, SR-90107, KFA-1411, RPR-120844, SEL-2489, SEL-2711, SEL-1915, SEL-2219, danaparoid, heparin, Factorex, ardeparin, CY-222, benzamido-benzodiazepinone derivatives, YM-75466, RPR-807834, BX-807834, ZK-805412, ZD-4927, antistatin, diarylsulfonamides, CVS-1578, CVS-1778, CVS-2097, BCH-1710, YM-60828, L-375378, desmin 370, CVS-1801, LY-368052, GR-133487, P-0933
Factor VIIa (3.4.21.21)	Thrombosis Deep vein thrombosis Disseminated intravascular coagulation Myocardial infarction Angina Lung embolism	Bikunin, NAP-B, NAPc2, plancinin, aprosulate, heparin
TNF-alpha converting enzyme (TACE) (3.4.24)	Arthritis Osteoporosis Inflammatory bowel disease Rheumatoid arthritis Ulcerative colitis	GW-1988, BB-2983, BB-3635, GW-3333, D-5410, CH-138, CH-175, CH-263, marimastat analogs
Beta-Lactamase (3.5.2.6)	Bacterial infections	Clavulanic acid, sulbactam, tazobactam, Ro-61-0480, HM-3030, GD-40, Ro-48-8724, Ro-48-1220, Syn-2190, SB-206999Z, LL-10G568-beta, CL-186659, CL-186195, DWC-751, 2085-P, CP-72436, BK-218, BRL-42715, SB-223328, Ro-47-1317
Undecaprenyldiphosphatase (3.6.1.27)	Bacterial infections	Bacitracin
H+/K+ ATPase (3.6.1.36)	Peptic ulcer GERD Gastrointestinal disease Bacterial infection Fungal infection Parasite infection	Omeprazole, leminoprazole, rabeprazole, egualen, SKF-97574, TU-199, GYKI-34655, Ro-18-5364, T-776, nicotinamide derivatives, pantoprazole, lansoprazole, SKF-96067, SPI-1447, AD-9161, YJA-20379, A-28200, SKF-96356, YM-020, bafilomycin A derivatives, atractylon, saviprazole, OPC-22575
Isomerase Enzymes		

DNA gyrase (5.99.1.3)	Bacterial infections Fungal infections Toxoplasmosis Parasite infections	Nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin, cinoxacin, sparfloxacin, lomefloxacin, fleroxacin, pefloxacin, amifloxiacin, novobiocin, cyclothialidine, DV-7751a, CJ-12371, CJ-12372, levofloxacin, moxifloxacin, trovafloxacin, A-104954, HSR-903, rufloxacin, KRQ-10018, clinafloxacin, A-101211, irloxacin, E-4749, tetracyclic thiazolopyridines, E-5065, E-4767, CP-99433, CP-67015, grepafloxacin, gatifloxacin, sitafloxacin, cetefloxacin, pazufloxacin, A-86719.1, E-4904, E-4884, E-4874, Y-26611, 773U82, U-91939, U-87947, A-65281, JP-9303, S-34109, A-990581, GR-122222X, MQ-0387, PGE-7119699, WQ-2743, DU-7751, ABT-255, WQ-3034, T-3811, SB-265805, DW-116, MF-5137, Wq-2128, CP-74667, Bay-y-3118, A-80556, A-74932, A-70826, PD-117596, PD-117558, Ro-23-9424, protosulfloxacin, S-31076, BMY-41889, BMY-41802, 7U85, temafloxacin, Ro-25-0993, Ro-25-1132, Ro-23-7777, DC-756, FA-103, NSFQ-105, CJ-12373, CJ-13136, CP-102372-1, LB-20277, PGE-9576326, WQ-2765, NM-394, Y-688, WQ-0835, A-3253, CS-940, PD-140248, PD-138312, PD-131628, BMY-43748, DB-289, tosulfloxacin, RG-201, annamycin, fostriecin
Topoisomerase II (5.99.1.3)	Cancer (testicular, lung, breast, Hodgkin's disease, non-Hodgkin's lymphoma, acute granylocytic leukemia, Kaposi's sarcoma, neoplasm, carcinoma, solid tumors)	Etoposide, teniposide, intoplicine, A-65281, teloxantrone, merbarone, elsamitrucin, indolium quaternary salts, fosquidone, pazelliptine, 7U85, mitonafide, CC-131, NK-109, NK-611, BE-13793C, NSC-665517, LU-125590, ER-37328, TAS-103, XR-5942, XR-5000, AD-347, AD-312, AHMA, ED-110, datelliptium, BE-10988, SR-103, morindone, NCA-0465, ICRF-193, TRK-710, TOP-53, Win-64593, CP-115953, losoxantrone, intoplicine, ellipticine, BO-2367, NSC-655649, AQ4N, aclacinomycin, NC-190, WR-63320, WIN-33377, CP-100964, GI-149893, elinafide, 773U82, Win-58161, merodantoin, BE-22179, BBR-2577, A-85266, clerocidin, SN-22995, A-74932 derivatives, W4R, azonafide, AMP-53, BBR-2828, Ro-47-3359, Ro-46-7864, Win-63320, azatoxin, IST-622, amrubicin, PD-131112, PD-115934, nemorubicin, A-74932, MPI-6003, HAR-7, sobuzoxane, S-16020-2, annamycin, asulacrine, GL-331, piroxantrone, iododoxorubicin, CI-958, adozelesin, mitoxantrone, DACA, fostriecin, cyclothialidine
Topoisomerase I (5.99.1.2)	Cancer (testicular, lung, breast, Hodgkin's disease, non-Hodgkin's lymphoma, acute granylocytic leukemia, Kaposi's sarcoma, neoplasm, cervix tumor, solid tumor, pancreas tumor, bladder tumor, carcinoma, head and neck tumor, colon tumor, colorectal tumor, prostate tumor, renal tumor, stomach tumor, glioma, myeloproliferative disorder, lymphoma)	Etoposide, teniposide, intoplicine, NB-506, lurotecan, 9-aminocamptothecin, elsamitrucin, RFS-2000, topotecan, indolium quaternary salts, TAN-1518A, NK-611, AG-555, BE-13793C, NSC-665517, LU-125590, TAS-103, XR-5942, J-107088, XR-5000, SKF-108025, ED-110, BN-80245, TRK-710, DX-8951, CP-115953, intoplicine, celastrol, julibrosides, A-35566-A, JSKIV-47, NSC-314622, NC-190, merocil, SKF-107874, SN-22995, NX-211, TAN-1496, azonafide, PD-115934, DU-6596, MPI-5019, HAR-7, NU/ICRF-505, NSC-675967, AP-4010, CKD-602, camptothecin, UCE-6, DACA, cyclothialidine
Ligase Enzymes		
Unclassified Enzymes		
Microsomal triglyceride transfer protein	Atherosclerosis Hyperlipidemia Hypercholesterolemia Hypertriglyceridemia	BMS-197636, BMS-200150, BMS-192951, BMS-201038, GR-328713, 4'-bromomethaqualone, 4'-bromo-3'-methylmethaqualone
UDP-GlcNAc transferase (2.4.2.30)	Bacterial infection	Ramoplanin

Those skilled in the art will appreciate that portions of the ligand structure that are not essential for molecular recognition and binding activity (i.e. that are not part of the ligand domain) may be varied substantially, replaced or substituted with unrelated structures (for example, with ancillary groups as defined below), and, in some cases, omitted entirely without

affecting the binding interaction. Accordingly, it should be understood that the term ligands is not intended to be limited to compounds known to be useful as enzyme inhibitors (for example, known drugs). Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not normally recognized as having useful properties related to enzyme binding. in that ligands that exhibit minimally useful properties as monomers can be highly active as multibinding agents, due to the biological benefit (increased biological effect) conferred by multivalency. The primary requirement for a ligand as defined herein is that it has a ligand domain as defined above.

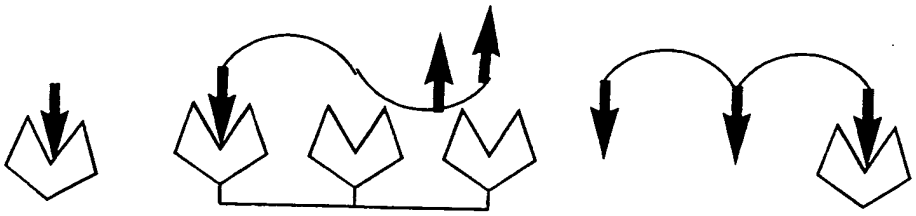
The preferred ligands fall within the following classes; glycopeptides, quinolones, azoles, beta-lactams, bacitracins, cyclase inhibitors, COX-2 inhibitors, statins, ACE inhibitors, phosphodiesterase inhibitors, aminoglycosides, tetracyclines, retroviral reverse transcriptase inhibitors, and vancomycin.

"Multibinding agent" or "multibinding compound" as used herein refers to a compound that is capable of multivalency as defined below, and which has 2-10 ligands, which may be the same or different, connected by one or more covalent linker or linkers, which may be the same or different, preferably from 1-20. A multibinding agent provides a biological and/or therapeutic effect greater than the aggregate of the unlinked ligands equivalent thereto. That is to say, an improved biological and/or therapeutic effect of the multibinding agent is obtained as measured against that achieved by the same number of unlinked ligands available for binding to the ligand binding site of the enzyme, enzyme substrate, or enzyme cofactor. Examples of increased biological and/or therapeutic effect with respect to the target include, for example, increased specificity, increased affinity, increased selectivity, increased potency, increased efficacy, increased therapeutic index, a change in the duration of action, decreased toxicity, decreased side effects, improved bioavailability, improved pharmacokinetics, improved activity spectrum, improved ability to kill cells (e.g. a fungus), and the like. The multibinding compounds of the invention will exhibit one or more of the foregoing effects.

"Potency" as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multibinding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined

under identical test conditions (e.g., in an in vitro or in vivo assay or in an appropriate animal model, such as a human patient. The finding that the multibinding agent produces an equivalent biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g., on a per weight, per mole, or per ligand basis) is indicative of enhanced potency.

"Univalency" as used herein refers to a single binding interaction between the ligand domain of one ligand as defined herein with the ligand recognition site of an enzyme, enzyme substrate, or enzyme cofactor as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibits univalency when only one ligand of that compound is interacting with a ligand binding site. Examples of univalent interactions are depicted below.



where the arrow represents a ligand domain and the indent represents the ligand binding site of an enzyme,

"Multivalency" as used herein refers to the concurrent binding of 2 to 10 linked ligands (which may be the same or different) and two or more corresponding ligand binding sites of an enzyme, an enzyme substrate, or enzyme cofactor (which may be the same or different). Accordingly, two ligands connected by a linker that bind concurrently to two ligand binding sites of an enzyme, enzyme substrate, or enzyme cofactor would be considered as a bivalent compound; similarly, three ligands thus connected provide a trivalent compound. An example of trivalent binding, illustrating a multibinding agent bearing three ligands is shown below.

It should be understood that all compounds that contain multiple copies of a ligand attached to a linker (or linkers) do not necessarily exhibit the phenomena of multivalency, i.e. that the improved biological and/or therapeutic effect of the multibinding agent is obtained as measured against that produced by the same number of unlinked ligands available for binding to a ligand binding site. For multivalency to occur, the ligand domains of the ligands that are connected by a linker have to be presented to their "receptors" (i.e. the ligand binding sites of the

enzyme, enzyme substrate, or enzyme cofactor) by the linker in a specific manner in order to bring about the desired ligand–orienting result, and thus produce a multibinding event. Thus, the term "multimeric ligand compound" refers to multiple copies of a ligand attached to a linker (or linkers) that may or may not exhibit the phenomena of multivalency. Although some enzyme-binding multimeric compounds are known in the literature (see, for example, J. Med. Chem. 1978, Vol. 21, 1132-1136, J. Med. Chem. 1995, Vol. 38, 1608-1628, Bioorganic and Medicinal Chemistry, Vol. 4 (1984), 653-656, U.S. Patent 5,171,864, WO 90/02129, and WO 96/19979), there is no general recognition of the phenomena of multibinding, or of methods for the identification of multibinding agents or compounds from a library of multimeric compounds. "Multimeric ligand compound library" refers to the collection of multimeric ligand compounds that are provided by the synthetic methods disclosed herein.

"Selectivity" or "specificity" is a measure of the binding preferences of a ligand for different receptors (enzymes, enzyme substrates, or enzyme cofactors) and/or different ligands for the same receptor. The selectivity of a ligand with respect to its target receptor relative to another receptor is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex), or in cases where a biological effect is observed below the K_d, selectivity is given by the ratio of the respective EC_{50}s (i.e. the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct receptors).

The term "ligand recognition site" or "ligand binding site" as used herein denotes the site on an enzyme that recognizes a ligand domain and provides a binding partner for a ligand, enzyme substrate, or enzyme cofactor. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example, agonism, antagonism, modulatory effect, and the like, or may maintain an ongoing biological effect. For the present purpose the ligand (or the ligand domain) and the ligand binding site cannot both be an antibody, an antibody domain, or a fragment of an antibody.

It should be recognized that the ligand binding sites of the enzymes that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations (e.g., enzymes may be covalently joined in a single structure, noncovalently associated in a multimeric structure, embedded in a membrane or polymeric matrix, and so on) and therefore have less relative translational and rotational freedom than if the

same enzymes were present as monomers in solution.

The terms "agonism" and "antagonism" are well known in the art. By the term "modulatory effect" we mean the ability of a ligand to change the biological effect of an agonist or antagonist through binding to a receptor (enzyme, enzyme substrate, or enzyme cofactor).

Enzymes, as categorized by The International Union of Biochemistry and Molecular Biology (IUBMB), include the following:

1.	Oxidoreductases	2.	Transferases	3.	Hydrolases
4.	Lyases	5.	Isomerases	6.	Ligases (synthetases)

1. Oxidoreductases catalyze reactions that involve the transfer of electrons between substrates and transition metal ions or organic cofactors. For example, sterol 14-alpha methyl demethylase, a heme-containing, membrane-associated enzyme, catalyzes the oxidative removal of a methyl group from lanosterol during the course of sterol biosynthesis in fungal and mammalian cells. The terminal acceptor of electrons in this reaction is the oxidized form of a nicotinamide-adenine dinucleotide cofactor. Specific inhibition of the fungal demethylase enzyme by azoles such as ketoconazole, itraconazole, and fluconazole forms the basis for effective antifungal therapy. A second example of an oxidoreductase enzyme is squalene monooxygenase, which is responsible for the conversion of squalene to (3*S*)-2,3-oxidosqualene in plant, fungal, and mammalian cells. This reaction is iron-dependent and requires dioxygen and a reduced flavin cofactor. Selective inhibition of the fungal enzyme by the allylamine family of inhibitors (including naftifine and terbinafine) is used for topical treatment of a variety of fungal infections. A third example of an oxidoreductase enzyme is cyclooxygenase (prostaglandin H2 synthase), which catalyzes the conversion of arachidonic acid to prostaglandin G2 and subsequently prostaglandin H2. There are two isoforms of this enzyme, the constitutively expressed COX-1 and COX-2, which is induced in inflammatory situations. These homodimeric, C2-symmetrical, membrane-associated enzymes are the targets of the non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin, indomethacin, ibuprofen, and naproxen. A fourth example of an oxidoreductase enzyme is dihydrofolate reductase (DHFR), which catalyzes the conversion of dihydrofolate polyglutamate to tetrahydrofolate polyglutamate using a reduced nicotinamide cofactor. DHFR is the primary site of action of antifolate cancer chemotherapeutics (e.g., methotrexate) and parasite chemotherapeutics (trimetrexate). A fifth example of an oxidoreductase is the homodimeric integral membrane enzyme hydroxymethyl

glutaryl-coenzyme A reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-determining step in sterol biosynthesis. Inhibition of this enzyme by the statins (e.g., lovastatin, atorvastatin) leads to reduction of serum cholesterol and triglyceride levels.

2. Transferase enzymes catalyze, among other things, the primary processes by which biological macromolecules are biosynthesized. DNA and RNA are formed through sequential phosphoryl group transfers, proteins are formed through sequential acyl group transfers, and polysaccharides are formed through sequential glycosyl group transfers. These processes constitute primary targets of a variety of anticancer and antimicrobial agents. As previously noted, the process catalyzed by retroviral reverse transcriptase is inhibited by enzyme-mediated incorporation of activated forms of nucleotide analogs such as azidothymidine (AZT) by the procedure known as substrate adulteration. In a second example, DNA-dependent RNA polymerase in mycobacterial cells is selectively inhibited by the antibacterial agent rifampin. In a third example, protein biosynthesis in bacterial cells is selectively inhibited by a variety of antibiotics (including gentamicin and other aminoglycosides, tetracycline, chloramphenicol, erythromycin and other macrolides, clindamycin) that target binding sites on the ribosome, a multicomponent ribonucleoprotein complex that catalyzes the energy- and mRNA-dependent transfer of amino acid subunits on aminoacyl tRNAs to growing polypeptide chains. In a fourth example, vancomycin and other glycopeptide antibiotics inhibit the multifunctional, membrane-bound transglycosylase responsible for the glycosyl transferase reaction which incorporates lipid intermediate II into the growing bacterial cell wall. Vancomycin acts via a substrate sequestration mechanism; i.e., it binds to and sequesters lipid intermediate II within a complex that is not recognized by the transglycosylase. In a fifth example, the large family of beta-lactam antibiotics inhibit the action of a series of penicillin binding proteins (PBPs), most notably multifunctional, membrane-bound transpeptidase enzymes that are responsible for cross-linking of the bacterial cell wall.

3. Hydrolase enzymes mediate particular subsets of transfer reactions in which moieties are transferred to a water molecule partner. These reactions are central to the metabolism of biological macromolecules including nucleic acids, proteins, and polysaccharides. Hydrolysis reactions are also important for metabolism of smaller molecules. For example membrane-bound undecaprenyldiphosphatase catalyzes the hydrolysis of undecaprenyl pyrophosphate to undecaprenyl phosphate, which is then available for reuse as the lipid carrier for intermediates

involved in bacterial cell wall biosynthesis. The topical antibacterial agent bacitracin inhibits the diphosphatase process by sequestering the substrate, analogous to the mode of action of vancomycin. In a second example, beta-lactamases are responsible for ring-opening hydrolytic inactivation of beta-lactam antibiotics. These soluble, monomeric enzymes are irreversibly acylated by clavulanic acid and sulbactam, which can be used as an adjunct to beta-lactam antibiotics in antibacterial therapy. A third example of a hydrolase enzyme is the aspartyl protease from HIV, which is a homodimer of 99 amino acid subunits that combines to form a single active site. A fourth example of a hydrolase enzyme is the homotetrameric viral neuraminidase, which catalyzes the hydrolytic removal of terminal sialic acid groups from polysaccharides on mammalian cells which serve as adhesion ligands for the virus. Inhibition of viral neuraminidase by agents such as GS4104 represents a promising approach to the prophylaxis and therapy of infections due to influenza virus.

4. Lyase enzymes catalyze the addition of groups to double bonds, or the removal of groups to form double bonds. Examples include carbonic anhydrase, aspartase (which catalyzes the removal of ammonia from aspartic acid to form fumaric acid), and 5-dehydroquinate dehydrase (which catalyzes the dehydrative conversion of 5-dehydroquinate to 5-dehydroshikimate).

5. Isomerases catalyze rearrangements in the covalent framework of a substrate without changing its formal composition. One example of an isomerase enzyme is the homodimeric, membrane-associated lanosterol synthase, which catalyzes the complex cyclization/rearrangement of oxidosqualene to the first carbocyclic intermediate in mammalian and fungal sterol biosynthesis pathways. A second example of an isomerase is thromboxane synthase, which catalyzes the conversion of prostaglandin H2 to thromboxane A2. This process is inhibited by dazoxiben and pirmagrel. A third example of an isomerase is alanine racemase, a soluble, monomeric bacterial enzyme that produces D-alanine for incorporation into the cell wall. A fourth example of an isomerase is the family of topoisomerase enzymes, which are responsible for relaxing superhelical stress in double-stranded DNA molecules as they undergo transcription and replication. These enzymes are important targets of antimicrobial agents, in particular the quinolones, which inhibit the alpha2beta2 tetrameric topoisomerase II (DNA gyrase) in bacterial cells, and also etoposide, and teniposide, which inhibit the mammalian topoisomerase II enzyme.

6. Ligase enzymes join two molecules concomitant with the cleavage of a phosphoric

anhydride linkage, most usually that of ATP. One example of a ligase enzyme is D-alanyl-D-alanine ligase, which mediates the ATP-dependent condensation of two D-alanine subunits as an early step in bacterial cell wall biosynthesis.

Nearly all known enzymes are proteins. As is true for all proteins, the structures of enzymes may be described at several different levels. The primary structure consists of an unbranched polypeptide chain derived from head-to-tail condensation of L-alpha-amino acids (in some instances other amino acids are incorporated). As such, enzymes have distinct amino termini and carboxyl termini. Hydrogen bonding, hydrophobic, and other interactions between and among neighboring amino acid residues typically occur within preferred conformations for the main chain atoms, affording secondary structural elements such as beta sheets and alpha helices. Specific clustering of secondary structural elements may be recognizable as supersecondary structures and/or as part of independently folded domain units of an enzyme, leading eventually to the tertiary structure, the "arrangement in space of all atoms in a single polypeptide chain or in covalently linked chains." (Fersht)

Non-covalent oligomerization of protein subunits in enzymes is common, and the organization of these subunits is referred to as the quaternary structure. A great variety of enzyme quaternary structures are possible through variation in the numbers and identities of subunits. The simplest enzyme quaternary structures are those wherein two identical polypeptide chains combine to form an enzyme with a single active site. An example of such a structure is the homodimeric HIV protease. Lanosterol synthase and prostaglandin H2 synthase are examples of homodimeric enzymes with two distinct ligand recognition sites. The simplest heteromeric structure is a heterodimer, exemplified by the alpha-beta structure of farnesyl-protein transferase, which is responsible for farnesylation of Ras and other proteins. A higher-order structure is exemplified by alpha2-beta2 heterotetrameric DNA topoisomerase II, whose quaternary structure and two active sites is logical with respect to the pseudo-C2 symmetry of its DNA substrate and the nicking/passing/closing reactions catalyzed by this enzyme. At the far end of quaternary structural complexity is the eukaryotic ribosome, which contains four distinct rRNA strands and up to 100 individual polypeptides.

Enzymes (and enzymatic processes) are multivalent in nature when they bear two or more ligand recognition sites associated through the covalent structure of the enzyme or through the formation of a quaternary structure. They may also be multivalent by virtue of having at

least one active site and at least one distinct non-substrate ligand-binding site associated through the covalent structure of the enzyme or through the formation of a quaternary structure, are associated with a common surface (e.g., a cell membrane), and/or utilize multivalent substrates (e.g., substrates bearing multiple transformable groups and/or substrates associated with a common surface). Although not wishing to be bound or restricted by any particular theory or proposed mechanism of action, it is believed that as a consequence of the interrelated structure of enzymes, the multibinding agents of the invention can be made by utilizing any ligand that is recognized by a multivalent enzyme, enzyme substrate, or enzyme cofactor.

As used herein, the terms "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform ("$CHCl_3$"), methylene chloride (or dichloromethane or "CH_2C1_2), diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, is opropanol, tert-butanol, dioxane, pyridine, and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert solvents.

"Pharmaceutically acceptable salt" means those salts which retain the biological effectiveness and properties of the compounds of Formula I, and which are not biologically or otherwise undesirable. The compounds of Formula I are capable of forming both acid and base salts by virtue of the presence of amino and carboxyl groups respectively.

1. Pharmaceutically acceptable base addition salts may be prepared from inorganic and organic bases. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, substituted amines including naturally-occurring substituted amines, and cyclic amines, including is opropylamine, trimethyl amine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, and N-ethylpiperidine. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example carboxylic acid amides, including carboxamides, lower alkyl carboxamides, di(lower alkyl) carboxamides, and the like.

2. Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid,

nitric acid, phosphoric acid and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

It should be understood that Formula I as drawn is intended to include racemic ligands L and racemic linker X, as well as the individual stereoisomers of the ligands and linkers, including enantiomers and non-racemic mixtures thereof. The scope of the invention as described and claimed encompasses the racemic forms of the ligands and linkers as well as the individual enantiomers and non-racemic mixtures thereof, and of the compounds of Formula I per se.

The term "treatment" as used herein covers any treatment of a condition or disease in an animal, particularly a mammal, more particularly a human, and includes:

(i) preventing the disease or condition from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it;

(ii) inhibiting the disease or condition, i.e. arresting its development;

(iii) relieving the disease or condition, i.e. causing regression of the condition; or.

(iv) relieving the conditions caused by the disease, i.e. symptoms of the disease.

The term "disease or condition which is alleviated by treatment with a multibinding agent" as used herein covers all conditions and disease states which are generally acknowledged in the art to be usefully treated with the ligands of the invention in general, and those disease states which have been found to be usefully treated by the specific multibinding agents of our invention, including the compounds of Formula I. The term covers prophylactic treatment as well as relief or regression of the disease. It also covers the treatment of conditions that are not necessarily considered as disease states, for example the use of multibinding agents as contraceptives, as pregnancy limiting agents, for the treatment of insomnia, treatment of obesity, and the like.

Such disease states include, but are not limited to, treatment of a mammal afflicted with pathogenic bacteria, in particular staphylococci (methicillin sensitive and resistant), streptococci (penicillin sensitive and resistant), enterococci (vancomycin sensitive and resistant), and *Clostridium difficile*, gram-negative organisms including *E. Coli*, autoimmune disorders (such as psoriasis, multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes, and the like), breast cancer and prostate cancer, disease states related to blood clotting, neurodegenerative

disorders such as Parkinson's disease, transplant rejection or cancer (e.g. T-cell leukemia or lymphoma), viral diseases such as AIDS (reverse transcriptase or protease enzymes), arthritis, metastatic cancer and tumor growth related to metalloprotease enzymes, stress and hypertension.

The term "therapeutically effective amount" refers to that amount of a multibinding agent, for example a compound of Formula I, that is sufficient to effect treatment, as defined above, when administered to a mammal or avian in need of such treatment. The therapeutically effective amount will vary depending on the subject and disease state being treated, the severity of the affliction and the manner of administration, and the like, and may be determined routinely by one of ordinary skill in the art.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thio, amino or carboxyl group. The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product. Protecting groups are disclosed in more detail in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis" 2nd Ed., 1991, John Wiley and Sons, N.Y.

Preferred removable amino blocking groups include conventional substituents such as t-butyoxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (FMOC), allyloxycarbonyl (ALOC) and the like, which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, *t*-butyl etc. which can be removed by mild conditions compatible with the nature of the product.

"Linker" or "linkers" as used herein, identified where appropriate by the symbol X, refers to a group or groups that covalently link(s) from 2-10 ligands (as defined herein) in a manner that provides a compound capable of multivalency. The linker is a ligand domain orienting entity that permits attachment of multiple copies of ligands (which may be the same or different) thereto. The extent to which multivalent binding is realized depends upon the efficiency with

which the linker that joins the ligands permits the ligand domains to be presented to the ligand recognition sites (on enzymes, enzyme substrates, and enzyme cofactors). Beyond presenting ligand domains for multivalent interactions with multivalent receptors (enzymes, enzyme substrates, or enzyme cofactors), the linker spatially constrains these interactions to occur within dimensions defined by the linker. Thus, the structural features of the linker (valency, geometry, orienting capabilities, size, flexibility, chemical composition) are features of multibinding agents that play an important role in determining their activities. The term linker, however, does not include solid inert supports such as beads, resins, glass particles, rods, fibres, and the like, but it should be understood that the multibinding compounds of the invention can be attached to a solid support if desired to provide, for example, a material useful for separation and purification processes (e.g. affinity chromatography).

The ligands are covalently attached to the linker or linkers using conventional chemical techniques, for example reaction between a carboxylic acid and an amine to form an amide, an amine and a sulfonyl halide to form a sulfonamide, an alcohol or phenol with an alkyl or aryl halide to form an ether, and the like.

The linker (or linkers) is attached to the ligand at a position such that the ligand domain is permitted to orient itself appropriately in order to bind to the ligand binding site. The term linker embraces everything that is not considered to be part of the ligand.

The relative orientation in which the ligand domains are displayed derives from the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable substitutions can be made on a ligand is typically based on prior knowledge of structure-activity relationships of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., from X-ray crystallography, NMR. Such positions and the synthetic methods for covalent attachment are well known in the art.

Suitable linkers are discussed below.

At present, it is preferred that the multibinding agent is a bivalent compound, in which two ligands are covalently linked. For example, a glycopeptide dimer may be constructed by linking any hydroxyl group, carboxyl group or amino group of a first glycopeptide ligand to any hydroxyl group, carboxyl group or amino group of a second glycopeptide.

"Biological effect" as used herein includes, but is not limited to, increased affinity,

increased selectivity, increased potency, increased efficacy, increased duration of action, decreased toxicity, and the like.

Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for selecting compounds that have enzyme multibinding properties from a library of multimeric compounds.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds. The only requirement for the ligands chosen is that they are capable of interacting with an enzyme(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property

through the process of multibinding compound formation; i.e., such ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short in vivo half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding

compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor agonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or are elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal agonist binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in momomeric form.

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands, which may be agonists or antagonists, that bind to common or different targets.

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically inocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available, one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding sites reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in cis- or trans-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in cis- or trans-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties

impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarizability, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed in vivo.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

Having chosen a set of n ligands (n being determined by the sum of the number of different attachment points for each ligand chosen) and m linkers by the process outlined above, a library of (n!)m candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalies on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial libary is prepared in the solution

phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values are determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. In vitro efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, can also be determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data are determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, are readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both in vitro and in vivo).

Second, ascertaining the structure of those compounds which exhibit multibinding properties are accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libaries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative

binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in order to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates,diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

COMPLEMENTARY BINDING CHEMISTRIES

First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-hydroxyamine
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_4$	amine
ketone	amine/$NaCNBH_4$	amine
amine	isocyanate	urea

Representative ligands for use in this invention include, by way of example, L-1 through L-3 as identified below:

L-1	Mevastatin
L-2	Simvastatin
L-3	Lovastatin

Or alternatively, lovastatin substituted at three different attachment points could be represented as L-1, L-2, and L-3.

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-3 above and the second ligand and linker is selected from the following:

L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-
L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-

L-1/X-109- L-1/X-110- L-1/X-111- L-1/X-112- L-1/X-113- L-1/X-114-
L-1/X-115- L-1/X-116- L-1/X-117- L-1/X-118- L-1/X-119- L-1/X-120-
L-1/X-121- L-1/X-122- L-1/X-123- L-1/X-124- L-1/X-125- L-1/X-126-
L-1/X-127- L-1/X-128- L-1/X-129- L-1/X-130- L-1/X-131- L-1/X-132-
L-1/X-133- L-1/X-134- L-1/X-135- L-1/X-136- L-1/X-137- L-1/X-138-
L-1/X-139- L-1/X-140- L-1/X-141- L-1/X-142- L-1/X-143- L-1/X-144-
L-1/X-145- L-1/X-146- L-1/X-147- L-1/X-148- L-1/X-149- L-1/X-150-
L-1/X-151- L-1/X-152- L-1/X-153- L-1/X-154- L-1/X-155- L-1/X-156-
L-1/X-157- L-1/X-158- L-1/X-159- L-1/X-160- L-1/X-161- L-1/X-162-
L-1/X-173- L-1/X-174- L-1/X-175- L-1/X-176- L-1/X-177- L-1/X-178-
L-1/X-179- L-1/X-180- L-1/X-181- L-1/X-182- L-1/X-183- L-1/X-184-
L-1/X-185- L-1/X-186- L-1/X-187- L-1/X-188- L-1/X-189- L-1/X-190-
L-1/X-191- L-1/X-192- L-1/X-193- L-1/X-194- L-1/X-195- L-1/X-196-
L-1/X-197- L-1/X-198- L-1/X-199- L-1/X-200- L-1/X-201- L-1/X-202-
L-1/X-203- L-1/X-204- L-1/X-205- L-1/X-206- L-1/X-207- L-1/X-208-
L-1/X-209- L-1/X-210- L-1/X-211- L-1/X-212- L-1/X-213- L-1/X-214-
L-1/X-215- L-1/X-216- L-1/X-217- L-1/X-218- L-1/X-219- L-1/X-220-
L-1/X-221- L-1/X-222- L-1/X-223- L-1/X-224- L-1/X-225- L-1/X-226-
L-1/X-227- L-1/X-228- L-1/X-229- L-1/X-230- L-1/X-231- L-1/X-232-
L-1/X-233- L-1/X-234- L-1/X-235- L-1/X-236- L-1/X-237- L-1/X-238-
L-1/X-239- L-1/X-240- L-1/X-241- L-1/X-242- L-1/X-243- L-1/X-244-
L-1/X-245- L-1/X-246- L-1/X-247- L-1/X-248- L-1/X-249- L-1/X-250-
L-1/X-251- L-1/X-252- L-1/X-253- L-1/X-254- L-1/X-255- L-1/X-256-
L-1/X-257- L-1/X-258- L-1/X-259- L-1/X-260- L-1/X-261- L-1/X-262-
L-1/X-263- L-1/X-264- L-1/X-265- L-1/X-266- L-1/X-267- L-1/X-268-
L-1/X-269- L-1/X-270- L-1/X-271- L-1/X-272- L-1/X-273- L-1/X-274-
L-1/X-275- L-1/X-276- L-1/X-277- L-1/X-278- L-1/X-279- L-1/X-280-
L-1/X-281- L-1/X-282- L-1/X-283- L-1/X-284- L-1/X-285- L-1/X-286-
L-1/X-287- L-1/X-288- L-1/X-289- L-1/X-290- L-1/X-291- L-1/X-292-
L-1/X-293- L-1/X-294- L-1/X-295- L-1/X-296- L-1/X-297- L-1/X-298-
L-1/X-299- L-1/X-300- L-1/X-301- L-1/X-302- L-1/X-303- L-1/X-304-
L-1/X-305- L-1/X-306- L-1/X-307- L-1/X-308- L-1/X-309- L-1/X-310-
L-1/X-311- L-1/X-312- L-1/X-313- L-1/X-314- L-1/X-315- L-1/X-316-
L-1/X-317- L-1/X-318- L-1/X-319- L-1/X-320- L-1/X-321- L-1/X-322-
L-1/X-323- L-1/X-324- L-1/X-325- L-1/X-326- L-1/X-327- L-1/X-328-
L-1/X-329- L-1/X-330- L-1/X-331- L-1/X-332- L-1/X-333- L-1/X-334-
L-1/X-335- L-1/X-336- L-1/X-337- L-1/X-338- L-1/X-339- L-1/X-340-
L-1/X-341- L-1/X-342- L-1/X-343- L-1/X-344- L-1/X-345- L-1/X-346-
L-1/X-347- L-1/X-348- L-1/X-349- L-1/X-350- L-1/X-351- L-1/X-352-
L-1/X-353- L-1/X-354- L-1/X-355- L-1/X-356- L-1/X-357- L-1/X-358-
L-1/X-359- L-1/X-360- L-1/X-361- L-1/X-362- L-1/X-363- L-1/X-364-
L-1/X-365- L-1/X-366- L-1/X-367- L-1/X-368- L-1/X-369- L-1/X-370-
L-1/X-371- L-1/X-372- L-1/X-373- L-1/X-374- L-1/X-375- L-1/X-376-
L-1/X-377- L-1/X-378- L-1/X-379- L-1/X-380- L-1/X-381- L-1/X-382-
L-1/X-383- L-1/X-384- L-1/X-385- L-1/X-386- L-1/X-387- L-1/X-388-
L-1/X-389- L-1/X-390- L-1/X-391- L-1/X-392- L-1/X-393- L-1/X-394-

	L-1/X-395-	L-1/X-396-	L-1/X-397-	L-1/X-398-	L-1/X-399-	L-1/X-400-
	L-1/X-401-	L-1/X-402-	L-1/X-403-	L-1/X-404-	L-1/X-405-	L-1/X-406-
	L-1/X-407-	L-1/X-408-	L-1/X-409-	L-1/X-410-	L-1/X-411-	L-1/X-412-
	L-1/X-413-	L-1/X-414-	L-1/X-415-	L-1/X-416-	L-1/X-417-	L-1/X-418-
	L-2/X-1-	L-2/X-2-	L-2/X-3-	L-2/X-4-	L-2/X-5-	L-2/X-6-
	L-2/X-7-	L-2/X-8-	L-2/X-9-	L-2/X-10-	L-2/X-11-	L-2/X-12-
	L-2/X-13-	L-2/X-14-	L-2/X-15-	L-2/X-16-	L-2/X-17-	L-2/X-18-
	L-2/X-19-	L-2/X-20-	L-2/X-21-	L-2/X-22-	L-2/X-23-	L-2/X-24-
	L-2/X-25-	L-2/X-26-	L-2/X-27-	L-2/X-28-	L-2/X-29-	L-2/X-30-
	L-2/X-31-	L-2/X-32-	L-2/X-33-	L-2/X-34-	L-2/X-35-	L-2/X-36-
	L-2/X-37-	L-2/X-38-	L-2/X-39-	L-2/X-40-	L-2/X-41-	L-2/X-42-
	L-2/X-43-	L-2/X-44-	L-2/X-45-	L-2/X-46-	L-2/X-47-	L-2/X-48-
	L-2/X-49-	L-2/X-50-	L-2/X-51-	L-2/X-52-	L-2/X-53-	L-2/X-54-
	L-2/X-55-	L-2/X-56-	L-2/X-57-	L-2/X-58-	L-2/X-59-	L-2/X-60-
	L-2/X-61-	L-2/X-62-	L-2/X-63-	L-2/X-64-	L-2/X-65-	L-2/X-66-
	L-2/X-67-	L-2/X-68-	L-2/X-69-	L-2/X-70-	L-2/X-71-	L-2/X-72-
	L-2/X-73-	L-2/X-74-	L-2/X-75-	L-2/X-76-	L-2/X-77-	L-2/X-78-
	L-2/X-79-	L-2/X-80-	L-2/X-81-	L-2/X-82-	L-2/X-83-	L-2/X-84-
	L-2/X-85-	L-2/X-86-	L-2/X-87-	L-2/X-88-	L-2/X-89-	L-2/X-90-
	L-2/X-91-	L-2/X-92-	L-2/X-93-	L-2/X-94-	L-2/X-95-	L-2/X-96-
	L-2/X-97-	L-2/X-98-	L-2/X-99-	L-2/X-100-	L-2/X-101-	L-2/X-102-
	L-2/X-103-	L-2/X-104-	L-2/X-105-	L-2/X-106-	L-2/X-107-	L-2/X-108-
	L-2/X-109-	L-2/X-110-	L-2/X-111-	L-2/X-112-	L-2/X-113-	L-2/X-114-
	L-2/X-115-	L-2/X-116-	L-2/X-117-	L-2/X-118-	L-2/X-119-	L-2/X-120-
	L-2/X-121-	L-2/X-122-	L-2/X-123-	L-2/X-124-	L-2/X-125-	L-2/X-126-
	L-2/X-127-	L-2/X-128-	L-2/X-129-	L-2/X-130-	L-2/X-131-	L-2/X-132-
	L-2/X-133-	L-2/X-134-	L-2/X-135-	L-2/X-136-	L-2/X-137-	L-2/X-138-
	L-2/X-139-	L-2/X-140-	L-2/X-141-	L-2/X-142-	L-2/X-143-	L-2/X-144-
	L-2/X-145-	L-2/X-146-	L-2/X-147-	L-2/X-148-	L-2/X-149-	L-2/X-150-
	L-2/X-151-	L-2/X-152-	L-2/X-153-	L-2/X-154-	L-2/X-155-	L-2/X-156-
	L-2/X-157-	L-2/X-158-	L-2/X-159-	L-2/X-160-	L-2/X-161-	L-2/X-162-
	L-2/X-173-	L-2/X-174-	L-2/X-175-	L-2/X-176-	L-2/X-177-	L-2/X-178-
	L-2/X-179-	L-2/X-180-	L-2/X-181-	L-2/X-182-	L-2/X-183-	L-2/X-184-
	L-2/X-185-	L-2/X-186-	L-2/X-187-	L-2/X-188-	L-2/X-189-	L-2/X-190-
	L-2/X-191-	L-2/X-192-	L-2/X-193-	L-2/X-194-	L-2/X-195-	L-2/X-196-
	L-2/X-197-	L-2/X-198-	L-2/X-199-	L-2/X-200-	L-2/X-201-	L-2/X-202-
	L-2/X-203-	L-2/X-204-	L-2/X-205-	L-2/X-206-	L-2/X-207-	L-2/X-208-
	L-2/X-209-	L-2/X-210-	L-2/X-211-	L-2/X-212-	L-2/X-213-	L-2/X-214-
	L-2/X-215-	L-2/X-216-	L-2/X-217-	L-2/X-218-	L-2/X-219-	L-2/X-220-
	L-2/X-221-	L-2/X-222-	L-2/X-223-	L-2/X-224-	L-2/X-225-	L-2/X-226-
	L-2/X-227-	L-2/X-228-	L-2/X-229-	L-2/X-230-	L-2/X-231-	L-2/X-232-
	L-2/X-233-	L-2/X-234-	L-2/X-235-	L-2/X-236-	L-2/X-237-	L-2/X-238-
	L-2/X-239-	L-2/X-240-	L-2/X-241-	L-2/X-242-	L-2/X-243-	L-2/X-244-
	L-2/X-245-	L-2/X-246-	L-2/X-247-	L-2/X-248-	L-2/X-249-	L-2/X-250-
	L-2/X-251-	L-2/X-252-	L-2/X-253-	L-2/X-254-	L-2/X-255-	L-2/X-256-

L-2/X-257- L-2/X-258- L-2/X-259- L-2/X-260- L-2/X-261- L-2/X-262-
L-2/X-263- L-2/X-264- L-2/X-265- L-2/X-266- L-2/X-267- L-2/X-268-
L-2/X-269- L-2/X-270- L-2/X-271- L-2/X-272- L-2/X-273- L-2/X-274-
L-2/X-275- L-2/X-276- L-2/X-277- L-2/X-278- L-2/X-279- L-2/X-280-
L-2/X-281- L-2/X-282- L-2/X-283- L-2/X-284- L-2/X-285- L-2/X-286-
L-2/X-287- L-2/X-288- L-2/X-289- L-2/X-290- L-2/X-291- L-2/X-292-
L-2/X-293- L-2/X-294- L-2/X-295- L-2/X-296- L-2/X-297- L-2/X-298-
L-2/X-299- L-2/X-300- L-2/X-301- L-2/X-302- L-2/X-303- L-2/X-304-
L-2/X-305- L-2/X-306- L-2/X-307- L-2/X-308- L-2/X-309- L-2/X-310-
L-2/X-311- L-2/X-312- L-2/X-313- L-2/X-314- L-2/X-315- L-2/X-316-
L-2/X-317- L-2/X-318- L-2/X-319- L-2/X-320- L-2/X-321- L-2/X-322-
L-2/X-323- L-2/X-324- L-2/X-325- L-2/X-326- L-2/X-327- L-2/X-328-
L-2/X-329- L-2/X-330- L-2/X-331- L-2/X-332- L-2/X-333- L-2/X-334-
L-2/X-335- L-2/X-336- L-2/X-337- L-2/X-338- L-2/X-339- L-2/X-340-
L-2/X-341- L-2/X-342- L-2/X-343- L-2/X-344- L-2/X-345- L-2/X-346-
L-2/X-347- L-2/X-348- L-2/X-349- L-2/X-350- L-2/X-351- L-2/X-352-
L-2/X-353- L-2/X-354- L-2/X-355- L-2/X-356- L-2/X-357- L-2/X-358-
L-2/X-359- L-2/X-360- L-2/X-361- L-2/X-362- L-2/X-363- L-2/X-364-
L-2/X-365- L-2/X-366- L-2/X-367- L-2/X-368- L-2/X-369- L-2/X-370-
L-2/X-371- L-2/X-372- L-2/X-373- L-2/X-374- L-2/X-375- L-2/X-376-
L-2/X-377- L-2/X-378- L-2/X-379- L-2/X-380- L-2/X-381- L-2/X-382-
L-2/X-383- L-2/X-384- L-2/X-385- L-2/X-386- L-2/X-387- L-2/X-388-
L-2/X-389- L-2/X-390- L-2/X-391- L-2/X-392- L-2/X-393- L-2/X-394-
L-2/X-395- L-2/X-396- L-2/X-397- L-2/X-398- L-2/X-399- L-2/X-400-
L-2/X-401- L-2/X-402- L-2/X-403- L-2/X-404- L-2/X-405- L-2/X-406-
L-2/X-407- L-2/X-408- L-2/X-409- L-2/X-410- L-2/X-411- L-2/X-412-
L-2/X-413- L-2/X-414- L-2/X-415- L-2/X-416- L-2/X-417- L-2/X-418-

L-3/X-1- L-3/X-2- L-3/X-3- L-3/X-4- L-3/X-5- L-3/X-6-
L-3/X-7- L-3/X-8- L-3/X-9- L-3/X-10- L-3/X-11- L-3/X-12-
L-3/X-13- L-3/X-14- L-3/X-15- L-3/X-16- L-3/X-17- L-3/X-18-
L-3/X-19- L-3/X-20- L-3/X-21- L-3/X-22- L-3/X-23- L-3/X-24-
L-3/X-25- L-3/X-26- L-3/X-27- L-3/X-28- L-3/X-29- L-3/X-30-
L-3/X-31- L-3/X-32- L-3/X-33- L-3/X-34- L-3/X-35- L-3/X-36-
L-3/X-37- L-3/X-38- L-3/X-39- L-3/X-40- L-3/X-41- L-3/X-42-
L-3/X-43- L-3/X-44- L-3/X-45- L-3/X-46- L-3/X-47- L-3/X-48-
L-3/X-49- L-3/X-50- L-3/X-51- L-3/X-52- L-3/X-53- L-3/X-54-
L-3/X-55- L-3/X-56- L-3/X-57- L-3/X-58- L-3/X-59- L-3/X-60-
L-3/X-61- L-3/X-62- L-3/X-63- L-3/X-64- L-3/X-65- L-3/X-66-
L-3/X-67- L-3/X-68- L-3/X-69- L-3/X-70- L-3/X-71- L-3/X-72-
L-3/X-73- L-3/X-74- L-3/X-75- L-3/X-76- L-3/X-77- L-3/X-78-
L-3/X-79- L-3/X-80- L-3/X-81- L-3/X-82- L-3/X-83- L-3/X-84-
L-3/X-85- L-3/X-86- L-3/X-87- L-3/X-88- L-3/X-89- L-3/X-90-
L-3/X-91- L-3/X-92- L-3/X-93- L-3/X-94- L-3/X-95- L-3/X-96-
L-3/X-97- L-3/X-98- L-3/X-99- L-3/X-100- L-3/X-101- L-3/X-102-
L-3/X-103- L-3/X-104- L-3/X-105- L-3/X-106- L-3/X-107- L-3/X-108-

	L-3/X-109-	L-3/X-110-	L-3/X-111-	L-3/X-112-	L-3/X-113-	L-3/X-114-
	L-3/X-115-	L-3/X-116-	L-3/X-117-	L-3/X-118-	L-3/X-119-	L-3/X-120-
	L-3/X-121-	L-3/X-122-	L-3/X-123-	L-3/X-124-	L-3/X-125-	L-3/X-126-
	L-3/X-127-	L-3/X-128-	L-3/X-129-	L-3/X-130-	L-3/X-131-	L-3/X-132-
5	L-3/X-133-	L-3/X-134-	L-3/X-135-	L-3/X-136-	L-3/X-137-	L-3/X-138-
	L-3/X-139-	L-3/X-140-	L-3/X-141-	L-3/X-142-	L-3/X-143-	L-3/X-144-
	L-3/X-145-	L-3/X-146-	L-3/X-147-	L-3/X-148-	L-3/X-149-	L-3/X-150-
	L-3/X-151-	L-3/X-152-	L-3/X-153-	L-3/X-154-	L-3/X-155-	L-3/X-156-
	L-3/X-157-	L-3/X-158-	L-3/X-159-	L-3/X-160-	L-3/X-161-	L-3/X-162-
10	L-3/X-173-	L-3/X-174-	L-3/X-175-	L-3/X-176-	L-3/X-177-	L-3/X-178-
	L-3/X-179-	L-3/X-180-	L-3/X-181-	L-3/X-182-	L-3/X-183-	L-3/X-184-
	L-3/X-185-	L-3/X-186-	L-3/X-187-	L-3/X-188-	L-3/X-189-	L-3/X-190-
	L-3/X-191-	L-3/X-192-	L-3/X-193-	L-3/X-194-	L-3/X-195-	L-3/X-196-
	L-3/X-197-	L-3/X-198-	L-3/X-199-	L-3/X-200-	L-3/X-201-	L-3/X-202-
15	L-3/X-203-	L-3/X-204-	L-3/X-205-	L-3/X-206-	L-3/X-207-	L-3/X-208-
	L-3/X-209-	L-3/X-210-	L-3/X-211-	L-3/X-212-	L-3/X-213-	L-3/X-214-
	L-3/X-215-	L-3/X-216-	L-3/X-217-	L-3/X-218-	L-3/X-219-	L-3/X-220-
	L-3/X-221-	L-3/X-222-	L-3/X-223-	L-3/X-224-	L-3/X-225-	L-3/X-226-
	L-3/X-227-	L-3/X-228-	L-3/X-229-	L-3/X-230-	L-3/X-231-	L-3/X-232-
20	L-3/X-233-	L-3/X-234-	L-3/X-235-	L-3/X-236-	L-3/X-237-	L-3/X-238-
	L-3/X-239-	L-3/X-240-	L-3/X-241-	L-3/X-242-	L-3/X-243-	L-3/X-244-
	L-3/X-245-	L-3/X-246-	L-3/X-247-	L-3/X-248-	L-3/X-249-	L-3/X-250-
	L-3/X-251-	L-3/X-252-	L-3/X-253-	L-3/X-254-	L-3/X-255-	L-3/X-256-
	L-3/X-257-	L-3/X-258-	L-3/X-259-	L-3/X-260-	L-3/X-261-	L-3/X-262-
25	L-3/X-263-	L-3/X-264-	L-3/X-265-	L-3/X-266-	L-3/X-267-	L-3/X-268-
	L-3/X-269-	L-3/X-270-	L-3/X-271-	L-3/X-272-	L-3/X-273-	L-3/X-274-
	L-3/X-275-	L-3/X-276-	L-3/X-277-	L-3/X-278-	L-3/X-279-	L-3/X-280-
	L-3/X-281-	L-3/X-282-	L-3/X-283-	L-3/X-284-	L-3/X-285-	L-3/X-286-
	L-3/X-287-	L-3/X-288-	L-3/X-289-	L-3/X-290-	L-3/X-291-	L-3/X-292-
30	L-3/X-293-	L-3/X-294-	L-3/X-295-	L-3/X-296-	L-3/X-297-	L-3/X-298-
	L-3/X-299-	L-3/X-300-	L-3/X-301-	L-3/X-302-	L-3/X-303-	L-3/X-304-
	L-3/X-305-	L-3/X-306-	L-3/X-307-	L-3/X-308-	L-3/X-309-	L-3/X-310-
	L-3/X-311-	L-3/X-312-	L-3/X-313-	L-3/X-314-	L-3/X-315-	L-3/X-316-
	L-3/X-317-	L-3/X-318-	L-3/X-319-	L-3/X-320-	L-3/X-321-	L-3/X-322-
35	L-3/X-323-	L-3/X-324-	L-3/X-325-	L-3/X-326-	L-3/X-327-	L-3/X-328-
	L-3/X-329-	L-3/X-330-	L-3/X-331-	L-3/X-332-	L-3/X-333-	L-3/X-334-
	L-3/X-335-	L-3/X-336-	L-3/X-337-	L-3/X-338-	L-3/X-339-	L-3/X-340-
	L-3/X-341-	L-3/X-342-	L-3/X-343-	L-3/X-344-	L-3/X-345-	L-3/X-346-
	L-3/X-347-	L-3/X-348-	L-3/X-349-	L-3/X-350-	L-2/X-351-	L-3/X-352-
40	L-3/X-353-	L-3/X-354-	L-3/X-355-	L-3/X-356-	L-3/X-357-	L-3/X-358-
	L-3/X-359-	L-3/X-360-	L-3/X-361-	L-3/X-362-	L-3/X-363-	L-3/X-364-
	L-3/X-365-	L-3/X-366-	L-3/X-367-	L-3/X-368-	L-3/X-369-	L-3/X-370-
	L-3/X-371-	L-3/X-372-	L-3/X-373-	L-3/X-374-	L-3/X-375-	L-3/X-376-
	L-3/X-377-	L-3/X-378-	L-3/X-379-	L-3/X-380-	L-3/X-381-	L-3/X-382-
45	L-3/X-383-	L-3/X-384-	L-3/X-385-	L-3/X-386-	L-3/X-387-	L-3/X-388-
	L-3/X-389-	L-3/X-390-	L-3/X-391-	L-3/X-392-	L-3/X-393-	L-3/X-394-

L-3/X-395-	L-3/X-396-	L-3/X-397-	L-3/X-398-	L-3/X-399-	L-3/X-400-
L-3/X-401-	L-3/X-402-	L-3/X-403-	L-3/X-404-	L-3/X-405-	L-3/X-406-
L-3/X-407-	L-3/X-408-	L-3/X-409-	L-3/X-410-	L-3/X-411-	L-3/X-412-
L-3/X-413-	L-3/X-414-	L-3/X-415-	L-3/X-416-	L-3/X-417-	L-3/X-418-

Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of formula I are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions that contain, as the active ingredient, one or more of the compounds of formula I above associated with one or more pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium

silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.1 mg to about 1 g, more usually about 1 to about 100 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or

pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices that deliver the formulation in an appropriate manner.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 19th Edition, 1995, the complete disclosure of which is hereby incorporated by reference. The composition or formulation to be administered will, in any event, contain a quantity of the active compound(s) in an amount effective to alleviate the symptoms of the subject being treated.

UTILITY

The multibinding agents of the invention are useful in medical treatments related to the modulation of enzymatic processes, and accordingly exhibit biological effects well known to

those skilled in the art. For examples of such activity, see the Background to the Invention.

The multibinding agents of the invention are also useful as diagnostic agents, research tools, and for various *in vitro* uses. In addition, they are also useful for treating diseases related to animal health, including avian uses, as insecticides, for treating parasitic conditions, and for agricultural applications, such as crop protection (for example, treatment of fungal diseases in crops). Additionally, they are useful in the preparation of affinity resins (for example, by attaching the compounds of Formula I to a bead or resin), and consequently may be used for purifying enzymes by affinity chromatography.

TESTING

The compounds of Formula I are useful in medical treatments and exhibit biological effects that can be demonstrated in routine tests well known to those skilled in the art. For example, tests for antibiotic activity is referenced and described in the fourth edition of "Antibiotics in Laboratory Medicine", by Victor Lorian, M.D., published by Williams and Wilkins. A table of suitable assays is included in the Appendix (Table 2), and include:

Acetylcholinesterase

The ability to inhibit acetylecholinesterase is measured spectrophotometrically by the method of Ellman et al Biochem Pharmacol. 7:88, 1961. Activity is measured in vivo by the ability to restore cholinergically deficient memory in the Dark Avoidance Assay as described in US Patent 4,631,286.

Monoamine oxidase

The monoamine oxidase inhibition in vivo is determined by administering the compounds to animals and sacrificing the animals as various times. The MAO inhibiting activity in the liver homogenates is measured according to the method described in Biochem. Pharmacol. 12 (1963) 1439-1441. The activity in the brain can be measured in brain homogenastes according to the method described in Biochem Pharmacol. 12 (1963) 1439-1441. Activity as an antidepressant can be evaluated by antagonism of reserpine (5mg/kg i.p.) induced hypothermia.

5-Lipoxygenase

The activity of the compounds in an in vitro inhibitory assay is determined using the method described in U.S. Patent 4,873,259. A rat peritoneal anaphylaxis model as described in U.S. Patent 4,873,259 is used to determine in vivo activity.

Thromboxane A2 synthetase

The biosynthesis of thromboxane A2 can be evaluated using platelet malondialdehyude production as described in U.S. Patent 4,963,573.

HMGCoA reductase

In vitro activity of potential inhibitors is tested using the method described by Beg et al. (1977) FEBS Letters 80:123-129 using enzymes prepared as described by Kleinsek et al (1977) PNAS 74:1431-1435. Alternatively a microsomal assays of HMG-CoA reductase can be performed using rat liver microsomal suspensions freshly prepared and assayed according to Ackerman et al., J. Lipid Res. 18:408-413, 1977.

In vitro cell culture methods is used to determine cholesterol biosynthesis. This method is described in U.S. Patent 4,739,073.

In Vivo cholesterol biosynthesis inhibition is determined using rats as described in U.S. Patent 4,739,073 . The radiolabeled sterols can be precipitated as described Sperry et al., J. Bio. Chem. 187, 97 (1950).

H/K ATPase

Compounds are tested for their ability to inhibit gastric acid secretion as described in U.S. Patent 4,255,431.

Dihydrofolate reductase

Anti-leukemia activity is monitored using L1210 leukemia according to the method of Hutchinson et al. J. Cancer Res. 22:57-72 (1962) and U.S. Patent 4,369,319.

Reverse transcriptase

Anti-viral activity is measured by determining the concentration required to cause a 50% reduction in viral plaques or alternatively to cause a 50% reduction in virus cytopathic effect as described in U.S. Patent 5, 075,445.

Cyclic AMP phosphodiesterase

CAMP inhibitors showing nootropic effects have their in vivo activity measured by:means well known in the art.

Type III cyclic AMP phophodiesterase

The in vivo usefulness of compounds as cardiotonic agents is demonstrated by causing a significant increase in contractile force in the is olated cat atria and papillary muscle procedure and in causing a significant increase in cardiac contractile force in the anesthetized dog

procedure with low or minimal changes in heart rate and blood pressure. These procedures are described in U.S. Patent 4,072,746. Alternatively, guinea pig heart muscles can be used to monitor contractile response and the effect of cardiac contractility in anesthetized dogs can be measured according to U.S. Patent 4,751,227. The effect on coronary and femoral blood flow and platelet aggregation can also be measured as described in U.S. Patent 4,751,227.

Thromboxane synthetase

The in vitro activity of the enzyme is measured by employing horse platelet microsomes treated with indomethacin as described in Needleman et al. Science 193:163, 1976. The assay as described by U.S. Patent 4,518,602 and a radioimmunoassay detection as described in Shibouta et al., Biochem. Pharmacol. 28 3601, 1979.

METHODS OF PREPARATION

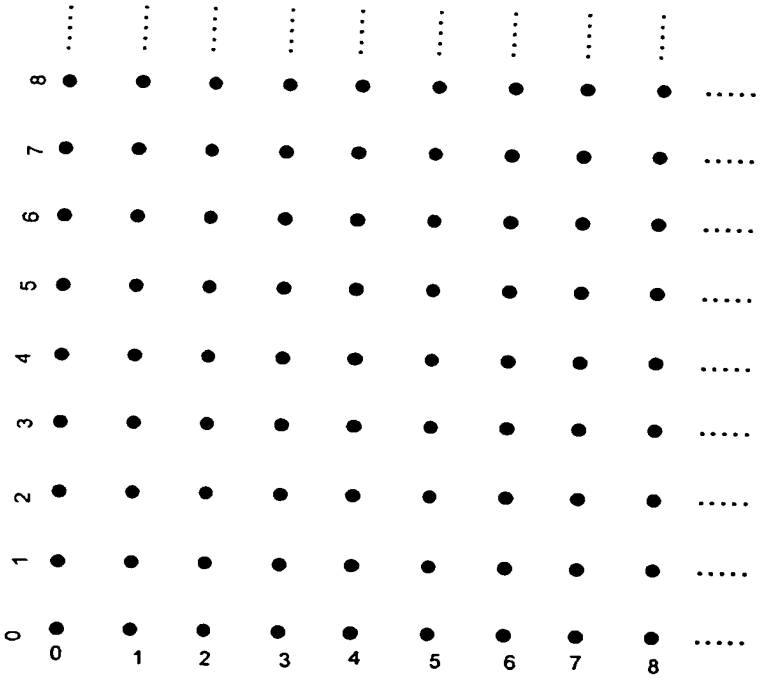
Linker

The linker (or linkers), when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multibinding agent. The biological effects of the multibinding agent are highly sensitive to the valency, geometry, composition, size, flexibility or rigidity, the presence or absence of anionic or cationic charge, and similar considerations (including hydrophilicity and hydrophobicity as discussed below) with respect to the linker. Accordingly, the linker is preferably chosen to maximize the desired biological effect. The linker may be biologically "neutral", i.e. not itself contribute any biological activity to the compound of Formula I, or it may be chosen to enhance the biological effect of the molecule. In general, the linker may be chosen from any organic molecule that orients two or more ligands to the receptors (enzymes, enzyme substrates, or enzyme cofactors), and permits multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand–orienting result, and thus produce a multibinding agent.

For example, different orientations can be achieved by including in the framework groups containing monocyclic or polycyclic groups, including aryl and heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Other

groups can also include oligomers and polymers which are branched- or straight-chain species. In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocycles, etc.). In other preferred embodiments, the ring is a six-or ten membered ring. In still further preferred embodiments, the ring is an aromatic group such as, for example, phenyl or naphthyl.

Different frameworks can be designed to provide preferred orientations of the ligands. Such frameworks may be represented by using an array of dots (as shown below) wherein each dot may potentially be an atom, such as C, O, N, S, P, H, F, Cl, Br, and F, or the dot may alternatively indicate the absence of an atom at that position. To facilitate the understanding of the framework structure, the framework is illustrated as a two dimensional array in the following diagram, although clearly the framework is a three dimensional array in practice:

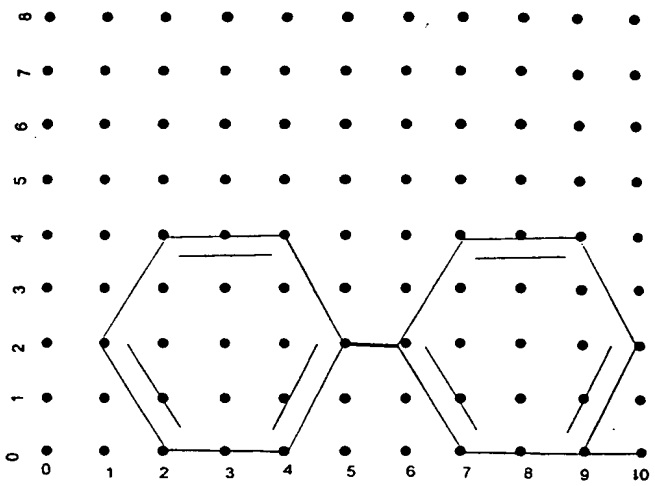


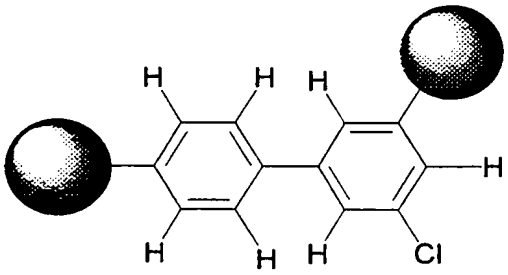
Each dot is either an atom, chosen from carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, or halogen, or the dot represents a point in space (i.e. an absence of an atom). Only certain atoms on the grid have the ability to act as an attachment point for the ligands, namely C, O, N, S, and P.

Atoms can be connected to each other via bonds (single, double, or triple with acceptable resonance and tautomeric forms), with regard to the usual constraints of chemical bonding.

Ligands may be attached to the framework via single, double, or triple bonds (with chemically acceptable tautomeric and resonance forms). Multiple ligand groups (2 to 10) can be attached to the framework such that the minimal, shortest path distance between adjacent ligand groups does not exceed 100 atoms or 40 angstroms.

An example of a linker as represented by the grid is shown below for a biphenyl construct.





Nodes (1,2), (2,0), (4,4), (5,2), (4,0), (6,2), (7,4), (9,4), (10,2), (9,0), (7,0) all represent carbon atoms. Node (10,0) is a chlorine atom. All other nodes (or dots) are points in space (i.e. represent an absence of atoms).

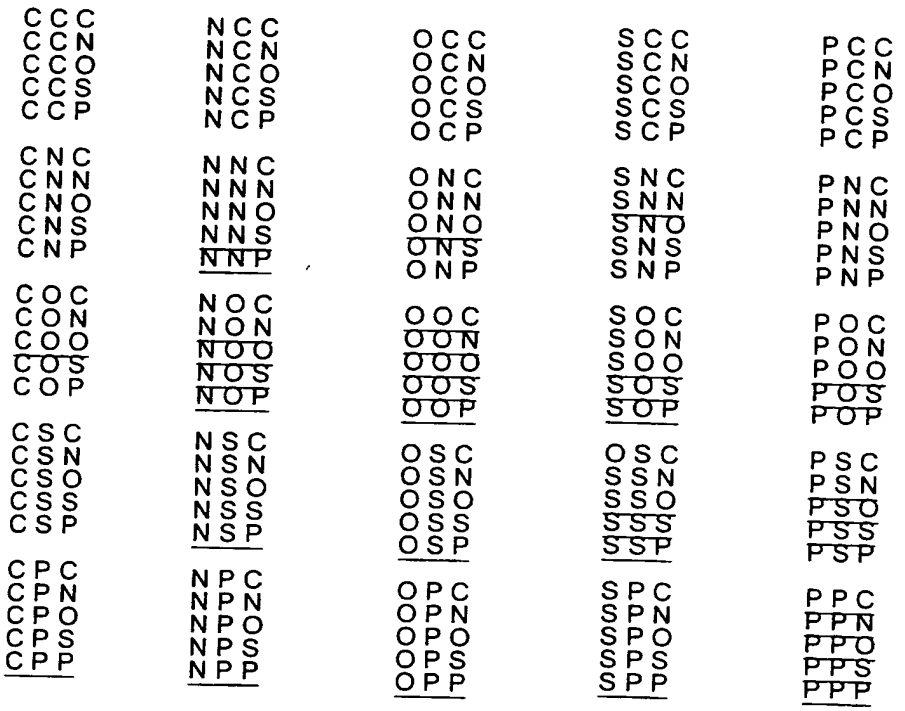
Nodes (1,2) and (9,4) are attachment points.

Hydrogen atoms are affixed to nodes (2,4), (4,4), (4,0), (2,0), (7,4), (10,2), and (7,0).

Nodes (5,2) and (6,2) are connected by a single bond.

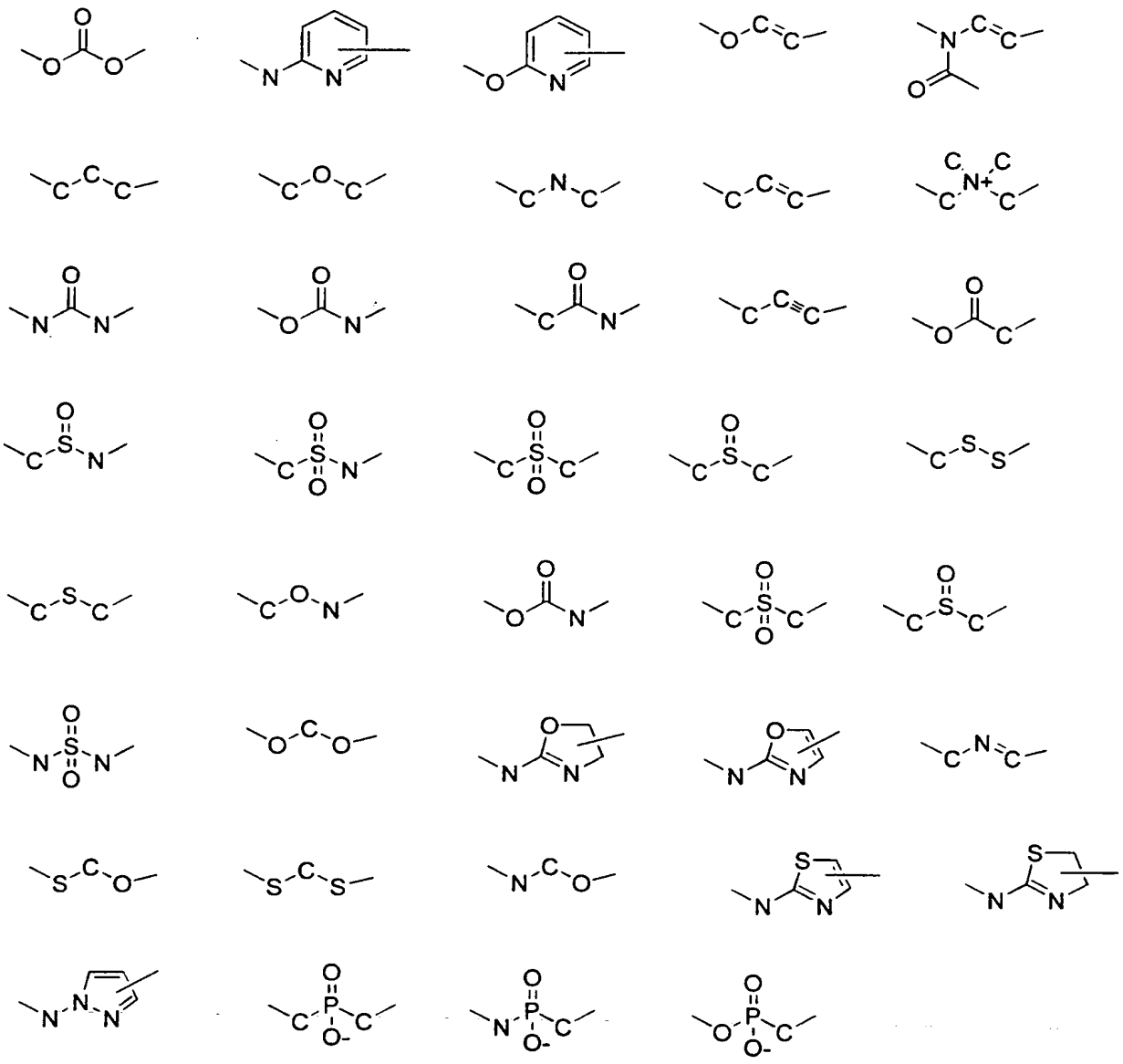
The carbon atoms present are connected by either single or double bonds, taking into consideration the principle of resonance and/or tautomerism.

The intersection of the framework (linker) and the ligand group, and indeed, the framework (linker) itself can have many different bonding patterns. Examples of acceptable patterns of three contiguous atom arrangements within the linker and at the linker-ligand interface are shown in the following diagram.



One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in "Advanced Organic Chemistry, 4th Edition" by March (Wiley-Interscience (New York), 1992). These arrangements are described in the grid of dots shown in the Scheme above. All of the possible arrangements for the five most preferred atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.



The identification of an appropriate framework geometry for ligand domain presentation is an important first step in the construction of a multivalent binding agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process. Various strategies are known to those skilled in the art of molecular design and can be used for preparing the compounds of this invention.

As shown in the Appendix (Examples of Dimeric Display), display vectors around similar central core structures can be varied, as can the spacing of the ligand domain from the core structure (i.e., the length of the attaching moiety). It is to be noted that core structures other

than those shown here, form example phenyldiacetylene and cyclohexane dicarboxylic acid, can be used for determining the optimal framework display orientation of the ligands. The process may require the use of multiple copies of the same central core structure or combinations of different types of display cores.

The above-described technique can be extended to trimers and compounds of higher-order valency as exemplified by structures shown in the Appendix.

Assay of each of the individual compounds of a collection generated as described above will lead to a subset of compounds with the desired enhanced activities (e.g., potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will provide a framework orientation that favors the properties desired. A wide diversity of linkers is commercially available (see, e.g., the Available Chemicals Directory, (ACD), Chem. Sources USA, Chem. Sources International, Chemical Abstracts). Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized, e.g., by methods known in the art and described below.

Having selected a preferred framework geometry, the physical properties of the linker can be optimized by varying the chemical composition. The composition of a linker can be varied in numerous ways to achieve the desired physical properties.

It can therefore be seen that there is a plethora of possibilities for the composition of a linker. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatic groups, ethers, lipids, cationic or anionic groups, or a combination thereof, and many specific examples of linkers are shown in the Appendix.

Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change solubility of the multibinding agent (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, molecular size, molecular weight, in vivo half-life, in vivo distribution, biocompatability, immunogenicity, stability, and the like. For example, the introduction of one or more poly or preferably oligo(ethylene glycol) (PEG) groups onto the linker enhances hydrophilicity and water solubility of the multibinding agent, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated

linker, may increase the in vivo retention time. Further, PEG may decrease antigenicity and potentially enhance the overall rigidity of the linker.

Ancillary groups that enhance the water solubility/hydrophilicity of the linker are useful in practicing the present invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, poly(ethylene glycol), alcohols, polyols (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligo- and polysaccharides, etc.), carboxylates, polycarboxylates (e.g., polyglutamic acid, polyacrylic acid, etc.), amines, polyamines (e.g., polylysine, poly(ethyleneimine), etc) to enhance the water solubility and/or hydrophilicity of the compounds of Formula I. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether. In particularly preferred embodiments, the ancillary group will be a poly(ethylene glycol).

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the compounds of Formula I is within the scope of the present invention. Lipophilic groups of use in practicing the instant invention include, but are not limited to, aryl and heteroaryl groups. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups of use in practicing the instant invention include fatty acid derivatives which do not form bilayers in aqueous medium until higher concentrations are reached.

Also within the scope of the present invention is the use of ancillary groups which result in the compound of Formula I being incorporated into a vesicle such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro, and other like groups. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or

dilinoleoylphosphatidylcholine could be used. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be reduced by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker or bonds between the linker and the ancillary group(s) or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the presence of rings and/or multiple bonds, for example, aryl, heteroaryl, cycloalkyl, and/or heterocyclic. Other groups which can impart rigidity include polymeric groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either negatively or positively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further, ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent bonding mechanism will tend to hold the linker into a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge which is unmasked, following addition to the linker, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those of skill in the art, is within the scope of the present invention.

Rigidity may also be imparted by internal hydrogen bonding, or by hydrophobic collapse.

Bulky groups can include, for example, large atoms and/or ions (e.g., iodine, sulfur, metal ions, etc.) groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocycles, etc.). In other preferred embodiments, the ring comprises one or

more six-membered rings. In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl.

Eliminating or reducing antigenicity of the compounds of Formula I by judicious choice of ancillary group(s) is within the scope of the present invention. In certain applications, the antigenicity of a compound of Formula I may be reduced or eliminated by the use of groups such as, for example, poly(ethylene glycol).

As explained above, the multibinding agents of the invention comprise 2-10 ligands attached to a linker that connects the ligands in such a manner that they are presented to the enzyme multivalent receptors for multivalent interactions with the appropriate receptors (ligand binding site). The linker spatially constrains these interactions to occur within dimensions defined by the linker, thus increasing the biological effect of the multibinding agent as compared to the same number of individual units of the ligand.

The multivalent compounds of the invention, the compounds of Formula I, are represented by the empirical formula $(L)_p(X)_q$. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is given below. However, as previously noted, the linker can be considered as a framework, and it should be understood that the ligands can be attached to this framework at any intermediate point on the framework, and/or on the termini of the framework. For example, if the linker is a linear chain, a bivalent compound can be constructed by attaching two ligands at the two ends of the linear chain, or alternatively attaching two ligands at some intermediate atom along the chain. The same considerations apply to the compounds of the present invention containing more than 2 ligands.

The simplest (and preferred) multibinding agent is a bivalent compound, which can be represented as L-X-L, where L is a ligand and is the same or different, and X is the linker. It should be noted that the linker X can be linear or cyclic, or a combination of both linear and cyclic constructs, and that the two ligands may be located at the termini of the linker or may be attached at some intermediate attachment point. This concept is diagrammed in the Appendix. The same is true for a trivalent compound, which can also be represented in a linear fashion, i.e. as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as can X, or a compound comprising three ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or

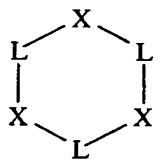
cycloalkyl group. See the Appendix for a pictorial representation of this concept, in which the shaded objects represent a ligand and the remaining structure represents the linker.

The same considerations of geometry apply to the compounds of the present invention containing 4-10 ligands. For example, a tetravalent compound could be represented as

L-X-L-X-L-X-L, or L-X-L-X-L
                  |
                  L

i.e. a branched construct analogous to the isomers of butane (n-butyl, sec-butyl, tert-butyl). Alternatively, it could be represented as an aryl or cycloalkyl derivative as above with four ligands attached to the core linker. The same principles apply to the higher multibinding agents, e.g. pentavalent to decavalent compounds. However, for multibinding agents attached to a central linker such as benzene, there is the self-evident constraint that there must be sufficient attachment sites on the linking moiety to accommodate the number of ligands present; for example, a benzene ring could not accommodate more than six ligands, whereas a saturated and/or multi-ring linker (cyclohexyl, cyclooctyl, biphenyl, etc.) could accommodate a larger number of ligands.

The formula $(L)_p(X)_q$ is also intended to represent a cyclic compound of formula $(-L-X-)_n$, where n is 2-10. For example, where n is 3:



All of the above variations are intended to be within the scope of the invention as defined by the Formula I $(L)_p(X)_q$.

The preferred linker length will vary depending upon the distance between adjacent ligand recognition sites, and the geometry, flexibility and composition of the linker. The length of the linker will preferably be in the range of about 2-100 Angstroms, more preferably about 2-50 Angstroms, and even more preferably about 5-20 Angstroms.

With the foregoing in mind, preferred linkers may be represented by the following formula:

$$-X'-Z-(Y'-Z)_m-Y''-Z-X'-$$

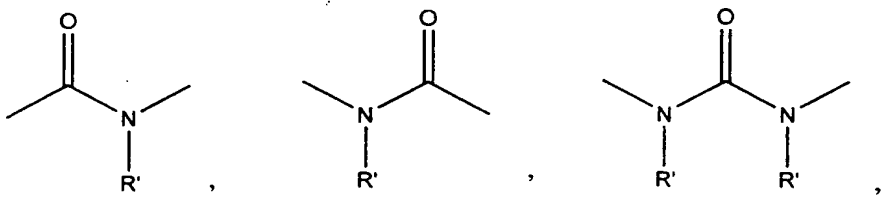
in which:

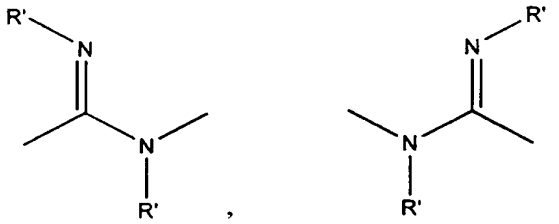
m is an integer of 0-20;

X' at each separate occurrence is –O-, -S-, -S(O)-, -$S(O)_2$-, –NR- (where R is as defined below), -C(O)-, or a covalent bond;

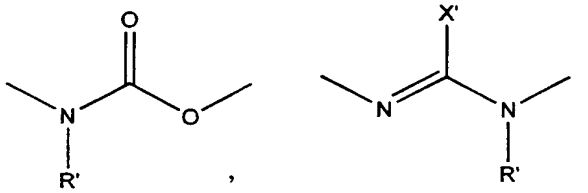
Z at each separate occurrence is alkylene, cycloalkylene, alkenylene, alkynylene, arylene, heteroarylene, or a covalent bond;

Y' and Y" at each separate occurrence are





-$P(O)_2(OR')$-O-,



-$S(O)_n$-CR'R"-, -$S(O)_n$-NR'-,

-S-S-, or a covalent bond;

in which:

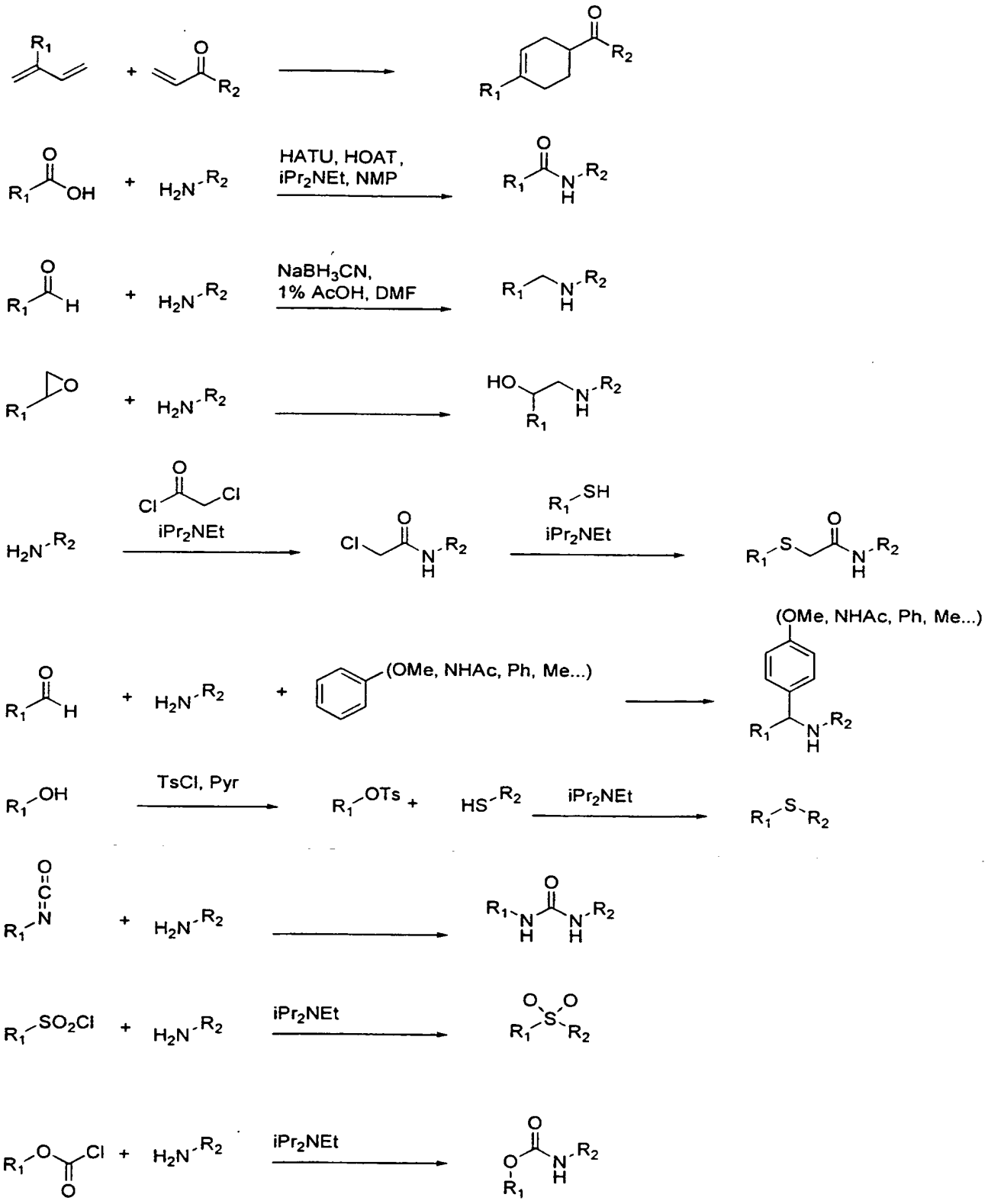
n is 0, 1 or 2; and

R, R' and R" at each separate occurrence are chosen from hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, and heterocyclo.

Additionally, the linker moiety can be optionally substituted at any atom in the chain by alkyl, cycloalkyl, alkenyl, alkynyl, alkoxy, halo, nitro, aryl, heteroaryl, or heterocyclo.

Preparation of Compounds of Formula I

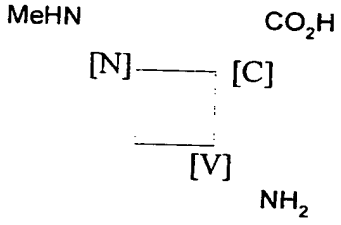
Examples of the chemistry for connecting ligands by a linker are shown in the table below, where R^1 and R^2 represent a ligand and/or the linking group.



Examples of the preparation of compounds of Formula I are shown below.

As indicated above, the simplest (and preferred) construct is a bivalent compound, which can be represented as L-X-L, where L is a ligand that is the same or different at each occurrence, and X is the linker.

Accordingly, an example of the preparation of a bivalent inhibitor of an enzymatic process is given below as an illustration of the manner in which multibinding agents of Formula I are obtained. This example is applicable to any ligand that includes amino and/or carboxyl groups, for example antibacterial agents such as vancomycin, teicoplanin, bacitracin, or quinolones such as nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin, and similar compounds. Examples of different linkers X are shown. In the reaction schemes that follow, for ease of understanding of the principles involved, the structure of the ligand is represented as a "box" with key functional groups included. Thus, the ligand is illustrated such that carboxyl [C], amino [V], and methylamino [N] groups are shown as examples of connecting points for the linker.



Two ligands are connected by the linker X via any carboxyl group or amino group of a first ligand to any carboxyl group or amino group of a second ligand.

Another simplification in the description of the preparations is that, for example, compound (1) is illustrated as a compound of formula H_2N-$(CH_2)_m$-$NHCO_2$-t-butyl, in which m is an integer of 1-20. However, it should be understood that $(CH_2)_m$ is not intended to signify or imply that the scope of this reaction (or of the invention) is limited to straight (i.e. unbranched) alkylene chains, but rather $(CH_2)_m$ is intended to include branched alkylenes as defined in the Detailed Description of the Invention, and alkylenes optionally substituted by aryl, arylalkyl, heteroaryl, heteroarylalkyl, heteroatoms, and the like, also as disclosed in the Detailed Description of the Invention. Similarly, the compound of formula (2) is illustrated as ClC(O)-$(CH_2)_n$-C(O)Cl, and $(CH_2)_n$ equally is not limited to straight alkylene chains, but includes all those modifications shown above..

Accordingly, bivalent compounds of Formula I where the linkage is from a [C] group of a first ligand to a [C] group of a second ligand, i.e. a [C-C] linkage, may be prepared from intermediates of formula (4), the preparation of which is shown below in Reaction Scheme 1.

REACTION SCHEME 1

$H_2N\text{-}(CH_2)_m\text{-}NHCO_2\text{-t-butyl}$ + $ClC(O)\text{-}(CH_2)_n\text{-}C(O)Cl$

(1) (2)

↓

t-butyl-$OC(O)\text{-}NH\text{-}(CH_2)_m\text{-}NH\text{-}C(O)\text{-}(CH_2)_n\text{-}C(O)\text{-}NH\text{-}(CH_2)_m\text{-}NH\text{-}C(O)$-t-butyl

(3)

↓

$H_2N\text{-}(CH_2)_m\text{-}NH\text{-}C(O)\text{-}(CH_2)_n\text{-}C(O)\text{-}NH\text{-}(CH_2)_m\text{-}NH_2$ (4)

in which m and n are independently at each occurrence integers of 1-20

Preparation of Compounds of Formula (3)

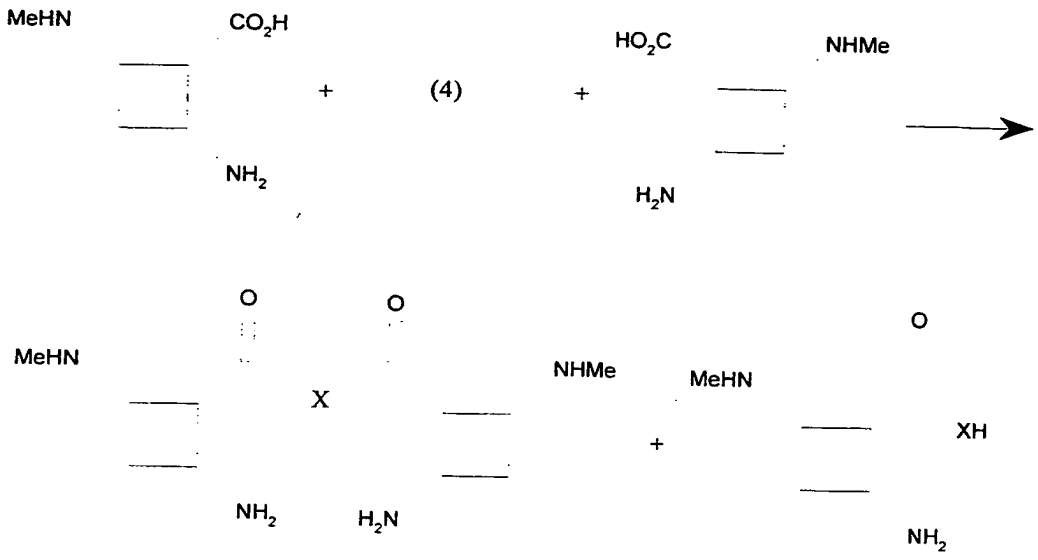
As illustrated in Reaction Scheme 1, step 1, about two molar equivalents of an omega-amino carbamic acid ester [formula (1)] is reacted with about one molar equivalent of a dicarboxylic acid halide, preferably chloride, of formula (2). The reaction is conducted in the presence of a hindered base, preferably diisopropylethylamine, in an inert solvent, preferably methylene chloride, at a temperature of about 0~5°C. The mixture is then allowed to warm to room temperature. When the reaction is substantially complete, the compound of formula (3) is isolated and purified by conventional means.

Preparation of Compounds of Formula (4)

As illustrated in Reaction Scheme 1, step 2, the carbamate is removed under acid conditions. In general a preferred acid is trifluoroacetic acid, and the reaction is conducted in an inert solvent, preferably methylene chloride, at about room temperature. When the reaction is substantially complete, the compound of formula (4) is isolated and purified by conventional means.

The compound of formula (4) is then converted into a [C-C] ligand dimer as shown in Reaction Scheme 2.

REACTION SCHEME 2



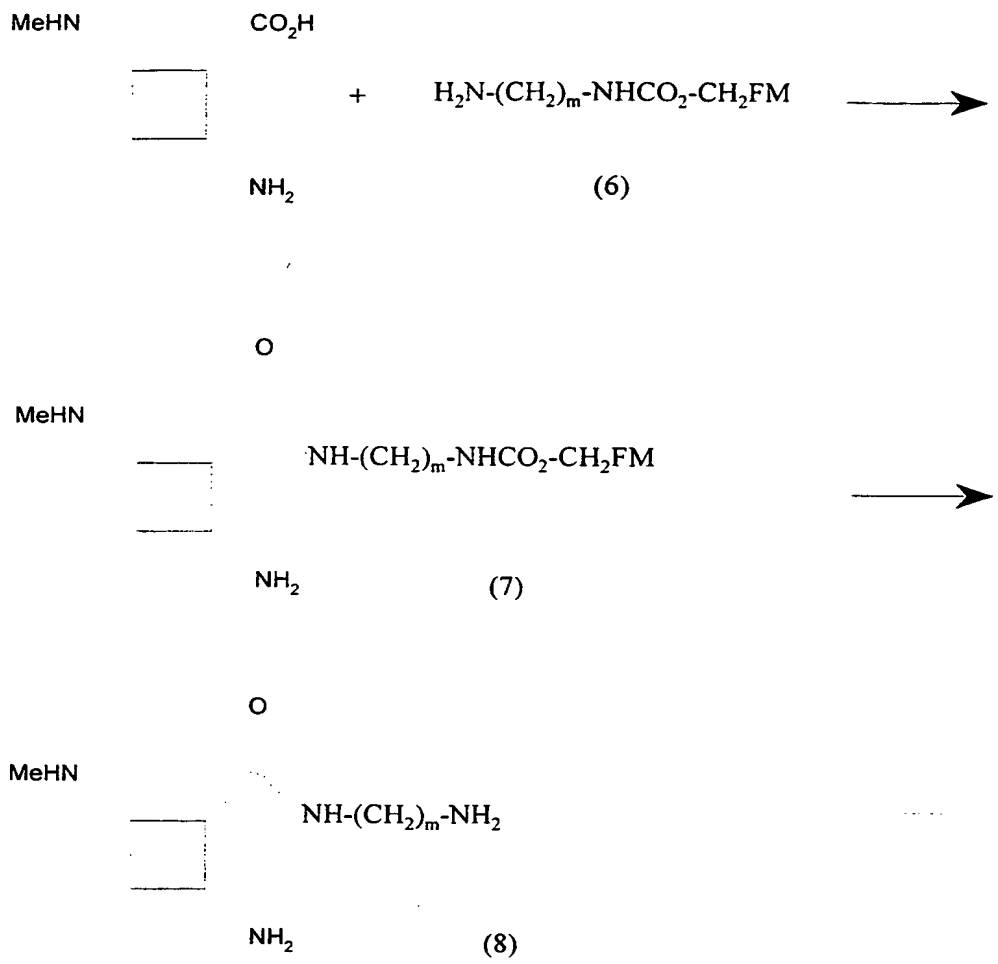
Formula I (5)

Preparation of Compounds of Formula I

In general, about two molar equivalents of ligand is reacted with about one molar equivalent of the compound of formula (4), under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, for example N,N-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO), or preferably a mixture of both, at about room temperature. When the reaction is substantially complete, the compound of Formula I is isolated and purified by conventional means, preferably purified by reverse phase HPLC. Also isolated is a byproduct of formula (5)

Alternatively, compounds of Formula I [C-C] may be prepared from intermediates of formula (8), the preparation of which is shown below in Reaction Scheme 3.

REACTION SCHEME 3



where FM represents 9-fluorenyl., and m is an integer of 1-20

Preparation of Compounds of Formula (7)

As illustrated in Reaction Scheme 3, step 1, ligand is reacted with about 1.1 molar equivalents of a carbamic ester terminated by an alkylamino group [formula (6)]. The ester moiety is chosen for ease of removal under mild conditions in subsequent reactions, and is preferably 9-fluorenylmethyl. Conventional amide coupling conditions are employed, preferably using PyBOP and l-hydroxybenzotriazole. In general, the reaction is conducted in the presence of a hindered base, preferably diisopropylethylamine, in an inert polar solvent, preferably DMF or DMSO, preferably a mixture of both, at about room temperature. When the reaction is substantially complete, the

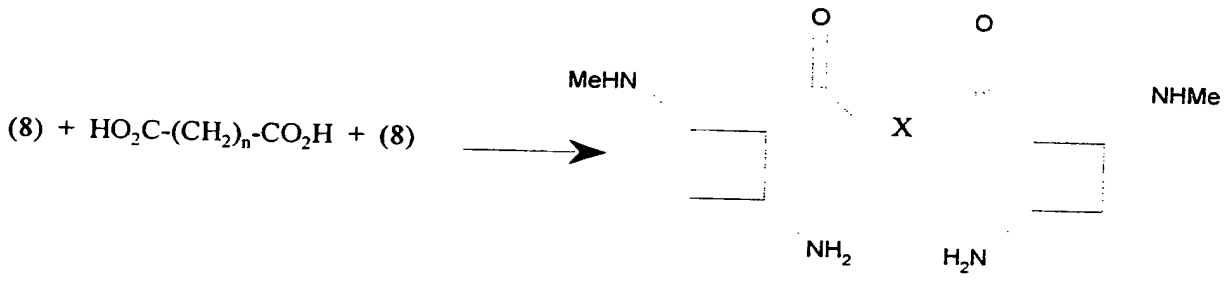
compound of formula (7) is isolated and purified by conventional means.

Preparation of Compounds of Formula (8)

As illustrated in Reaction Scheme 3, step 2, the compound of formula (7) is reacted with a mild base to remove the protecting ester group, which also affords decarboxylation. In general, the base is preferably piperidine, and the reaction is conducted in an inert polar solvent, preferably dimethylformamide, at about room temperature for about 10 minutes to one hour. When the reaction is substantially complete, the compound of formula (8) is isolated and purified by conventional means, preferably using reverse phase HPLC.

The compound of formula (8) is then converted into a [C-C] ligand dimer as shown in Reaction Scheme 4.

REACTION SCHEME 4



Formula I

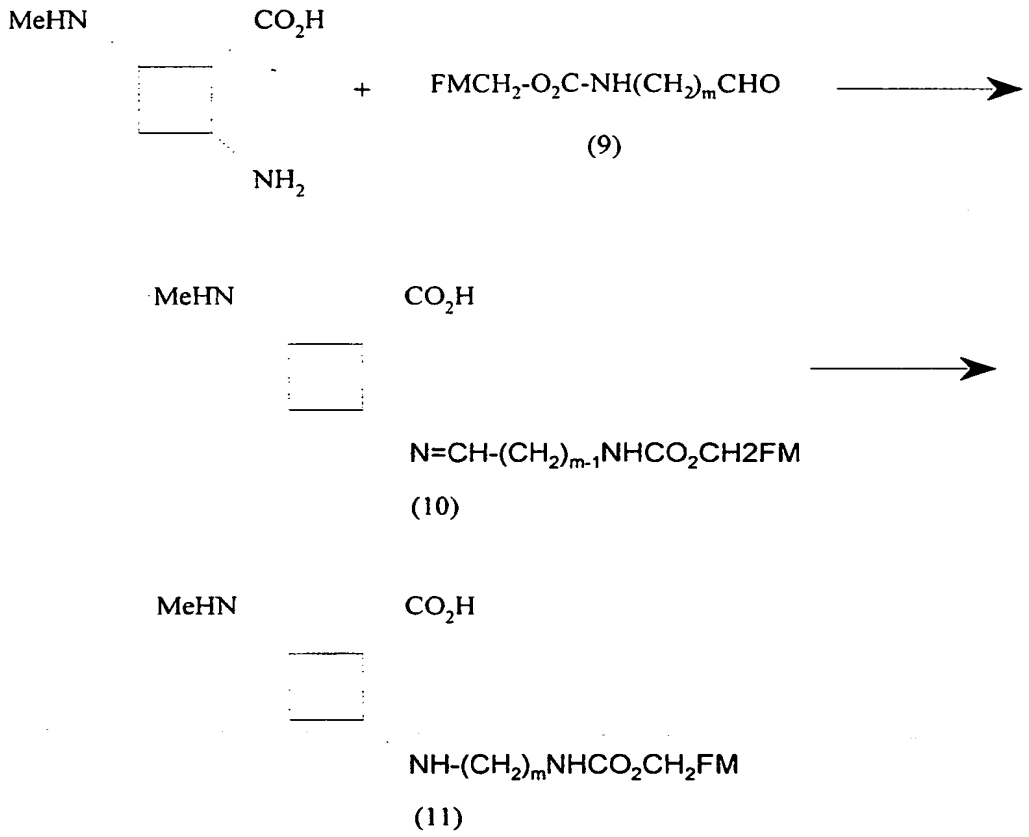
Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 4, the compound of formula (8) is reacted with a dicarboxylic acid. In general, about 3 molar equivalents of the compound of formula (8) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula HO_2C-$(CH_2)_n$-CO_2H, under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of PyBOP and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 1-3 hours. When the reaction is substantially complete, the compound of Formula I is isolated and purified by

conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [V-V] may be prepared from intermediates of formula (14), the preparation of which is shown below in Reaction Scheme 6. The starting material, the compound of formula (11), is prepared as shown in Reaction Scheme 5.

REACTION SCHEME 5



in which m is an integer of 1-20, and FM is 9-fluorenyl.

Preparation of Compounds of Formula (10)

As illustrated in Reaction Scheme 5, step 1, ligand having an $-NH_2$ group suitable for linking is reacted with a protected ester-aldehyde of formula (9) to form a Schiff's base. The ester moiety is chosen for ease of removal under mild conditions in subsequent reactions, and is preferably 9-

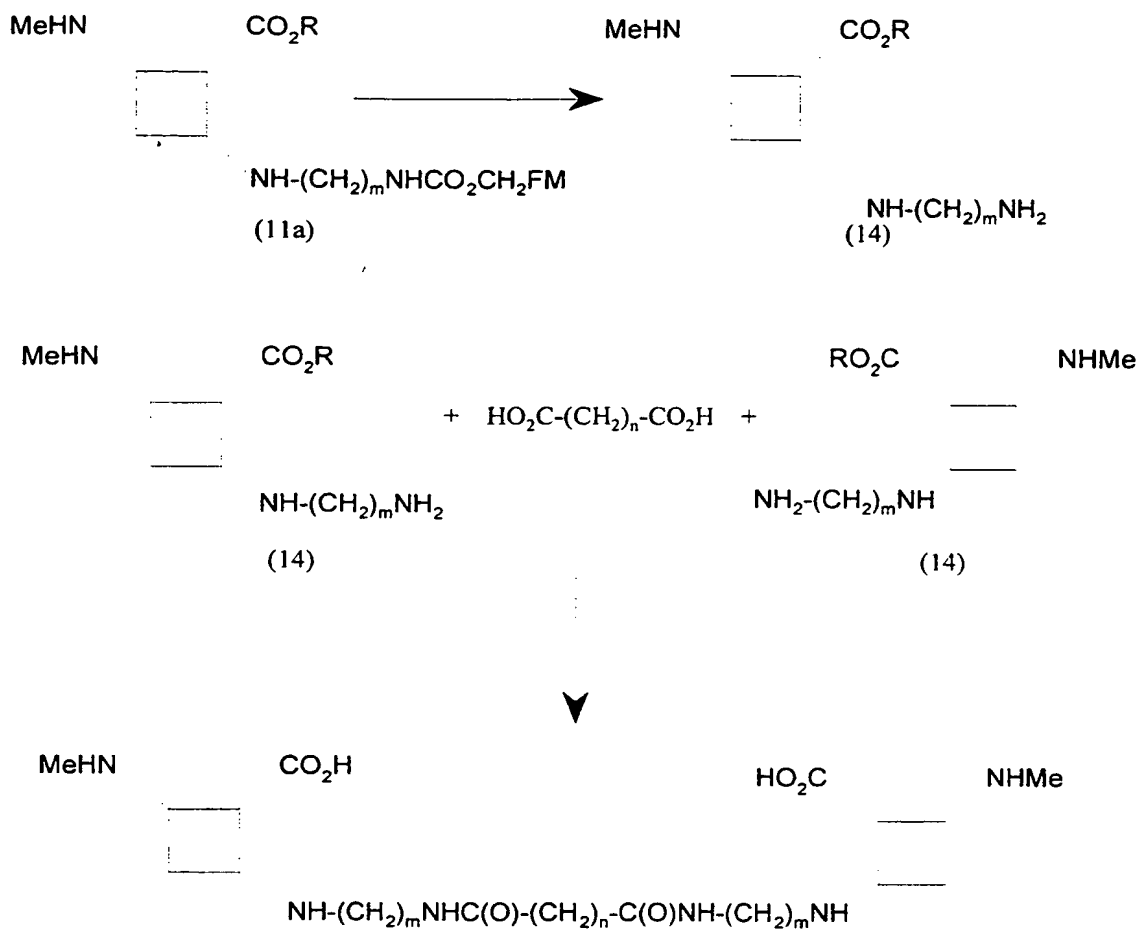
fluorenylmethyl. In general, the reaction is conducted in an inert polar solvent, preferably 3-dimethyl-3,4,5,6-tetrahydro~2(1H)-pyrimidinone plus methanol, at about 50-100°C, preferably about 70°C, for about 30 minutes to 1 hour. The Schiff's base of formula (10) is not isolated, but reacted further immediately as shown below.

Preparation of Compounds of Formula (11)

As illustrated in Reaction Scheme 5, step 2, the solution of the compound of formula (10) is further reacted with a mild reducing agent. In general, the reducing agent is preferably sodium cyanoborohydride, and the reaction is conducted at about 50~100°C, preferably about 70°C, for about 1-3 hours, preferably about 2 hours. When the reaction is substantially complete, the compound of formula (11) is isolated and purified by conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [V-V] may then be prepared from intermediates of formula (11a), the preparation of which is shown below in Reaction Scheme 6.

REACTION SCHEME 6



Formula I

where R is a protecting group, such as an ester, m and n are as defined above, and FM is 9-fluorenyl

Preparation of Compounds of Formula (14)

As illustrated in Reaction Scheme 6, step 1, the compound of formula (11a), which is a compound of formula (11) in which the carboxyl group has been protected conventionally, for example as an ester, is reacted with a mild base to remove the carbamate. In general, the base is preferably piperidine, and the reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 10 minutes to one hour, preferably about 30 minutes. When the

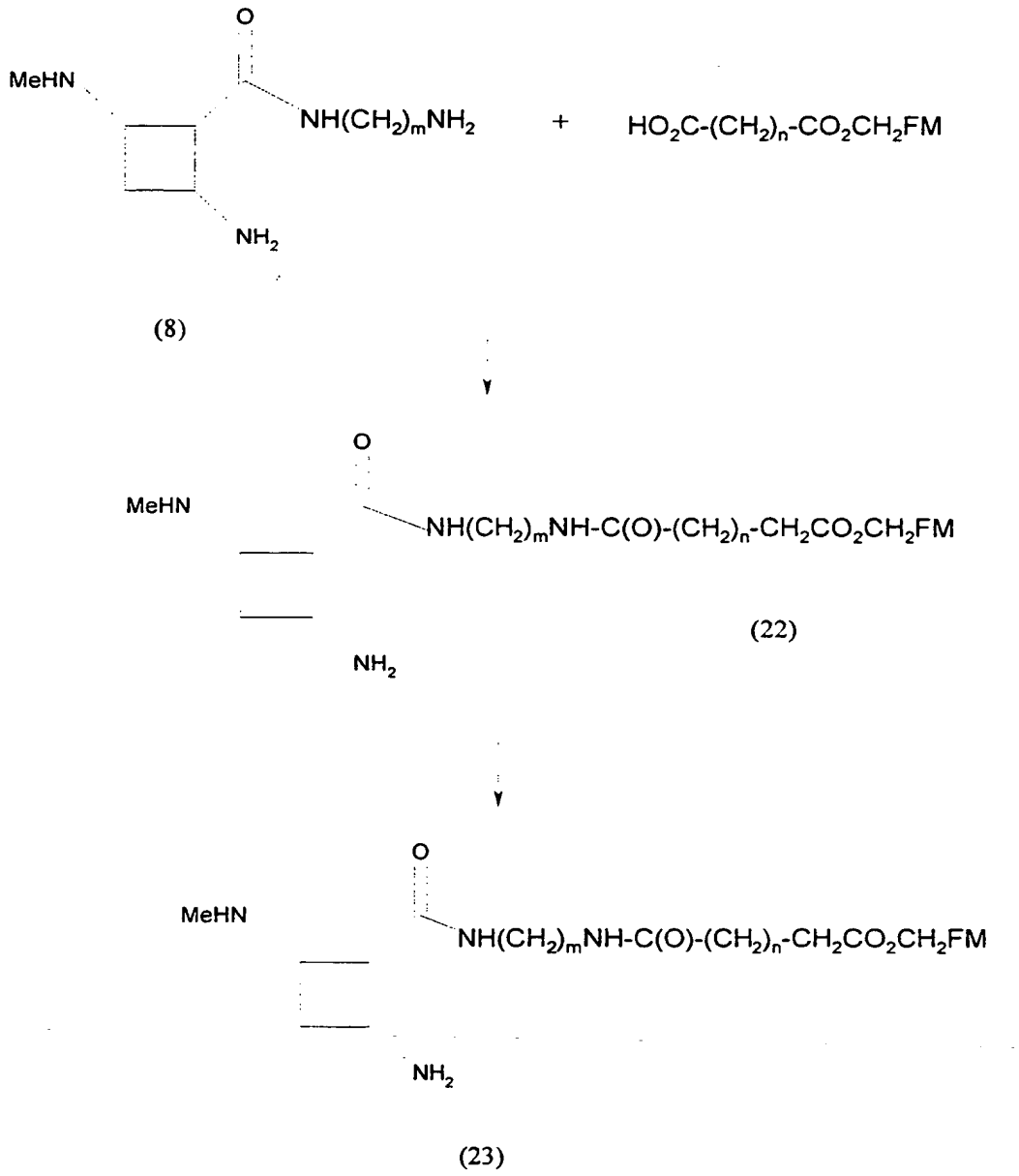
reaction is substantially complete, the compound of formula (14) is isolated and purified by conventional means, preferably using reverse phase HPLC.

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 6, step 2, the compound of formula (14) is reacted with a dicarboxylic acid. In general, about 3 molar equivalents of the compound of formula (8) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula HO_2C-(CH_2)n-CO_2H, under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of PyBOP and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 1-3 hours. When the reaction is substantially complete, the protecting group R, preferably an ester, is removed conventionally, and the [V-V] compound of Formula I is isolated and purified by conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [C-V] may be prepared from intermediates of formula (23), the preparation of which is shown below in Reaction Scheme 7. The starting material, the compound of formula (8), is prepared as previously shown.

REACTION SCHEME 7



Preparation of Compounds of Formula (22)

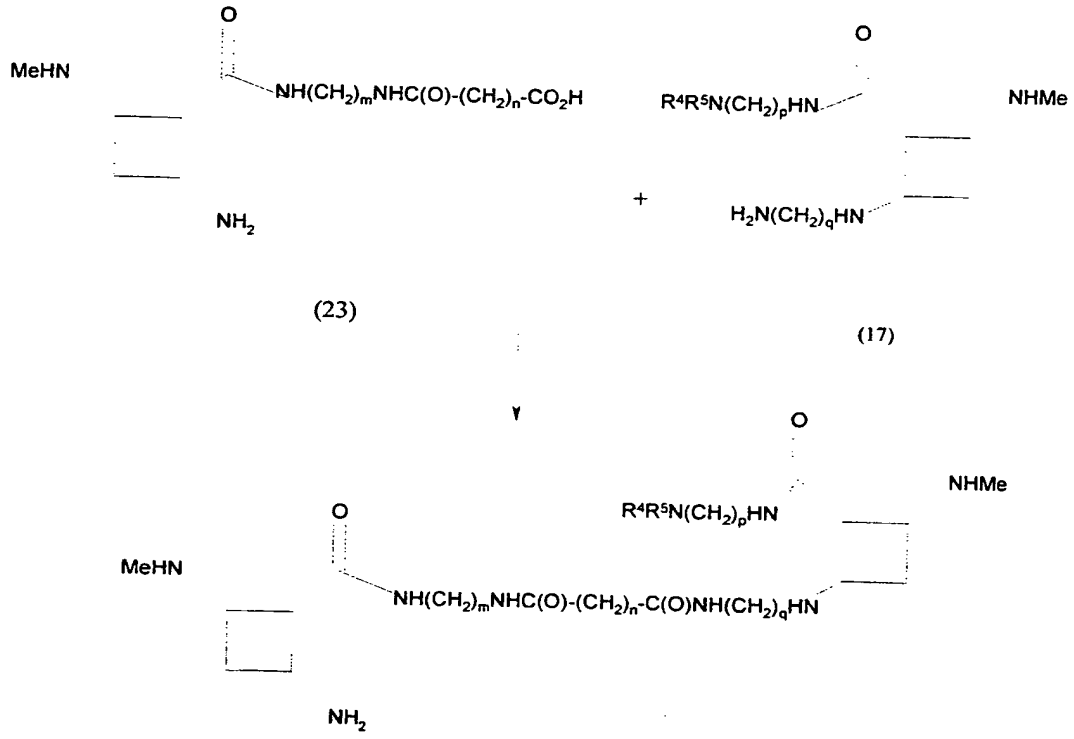
As illustrated in Reaction Scheme 7, step 1, the compound of formula (8) is reacted with an acid in the same manner as shown above, for example in Reaction Scheme 6, to form an amide of formula (22).

Preparation of Compounds of Formula (23)

As illustrated in Reaction Scheme 7, step 2, the compound of formula (22) is hydrolyzed with an base, for example piperidine, in the same manner as shown above, to form a compound of formula (23).

The compound of formula (23) is then converted into a [C-V] dimer of Formula I by reaction with a compound of formula (17), prepared as shown previously, as shown in Reaction Scheme 8.

REACTION SCHEME 8



Formula I

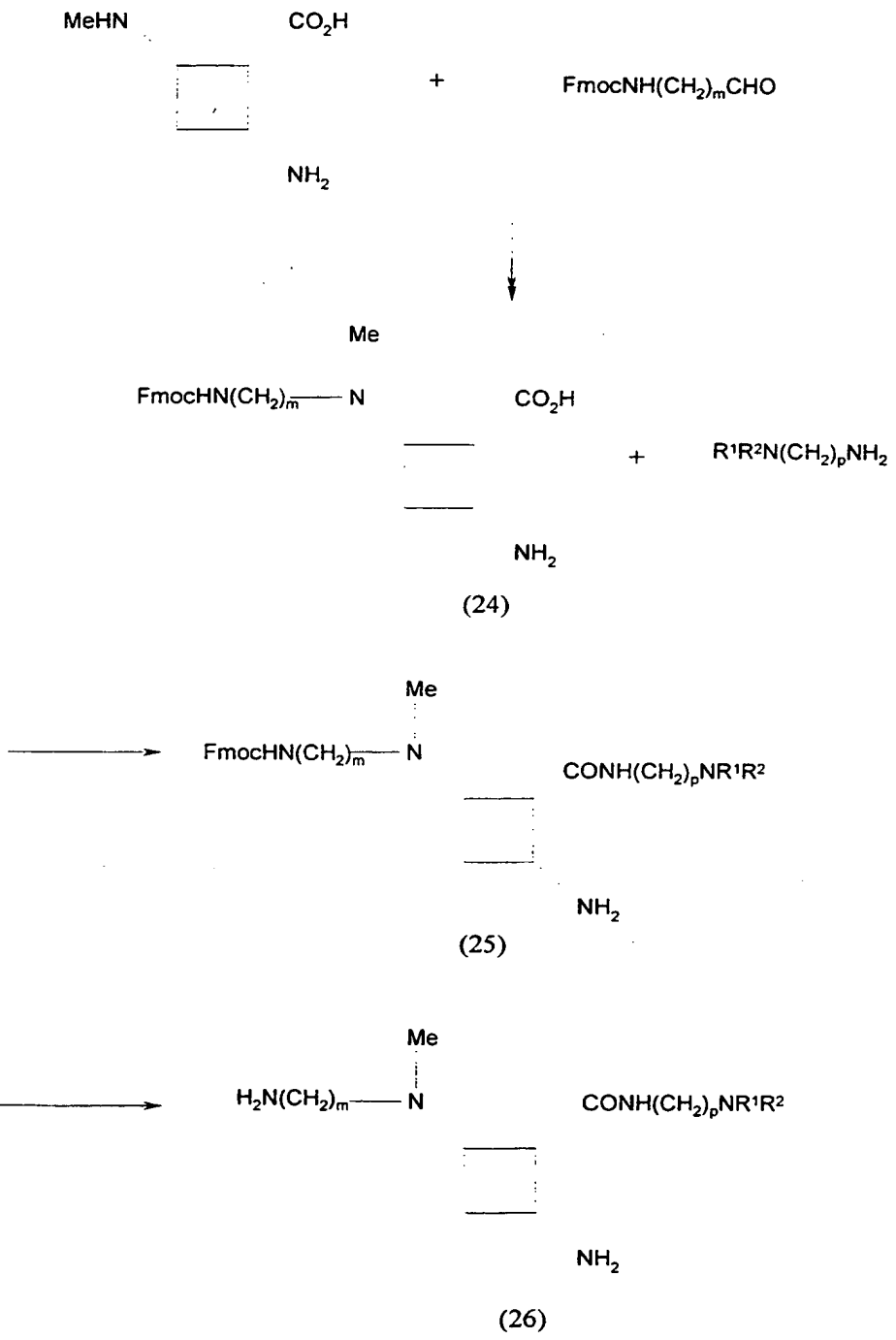
Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 8, the compound of formula (23) is reacted with a compound of formula (17) in a typical coupling reaction as shown above, to give a compound of Formula I [C-V].

Compounds of Formula I wherein the linkage is [C-N] may be prepared from intermediates

of formula (26), the preparation of which is shown below in Reaction Scheme 9

REACTION SCHEME 9



Preparation of Compounds of Formula (24)

As illustrated in Reaction Scheme 9, step 1, ligand is reacted with a protected aminoaldehyde in the presence of an amount of base sufficient to direct the reaction of the aldehyde to the [N] position. The Schiff's base thus formed is reduced in the same manner as shown in Reaction Scheme 5 to form a compound of formula (24).

Preparation of Compounds of Formula (25)

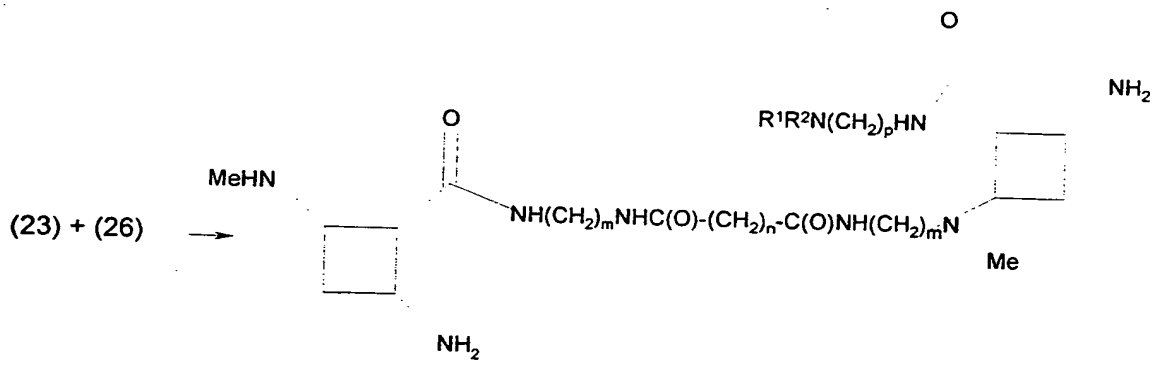
As illustrated in Reaction Scheme 9, step 2, the compound of formula (24) is reacted with an amine in a coupling reaction in the same manner as shown above, for example in Reaction Scheme 10, to form an amide of formula (25).

Preparation of Compounds of Formula (26)

As illustrated in Reaction Scheme 9, step 3, the protecting group FM is removed conventionally from the compound of formula (25) with a mild base to form a compound of formula (26).

The compound of formula (26) is then converted into a [C-N] dimer of Formula I by reaction with a compound of formula (23), prepared as shown previously, as shown in Reaction Scheme 10.

REACTION SCHEME 10



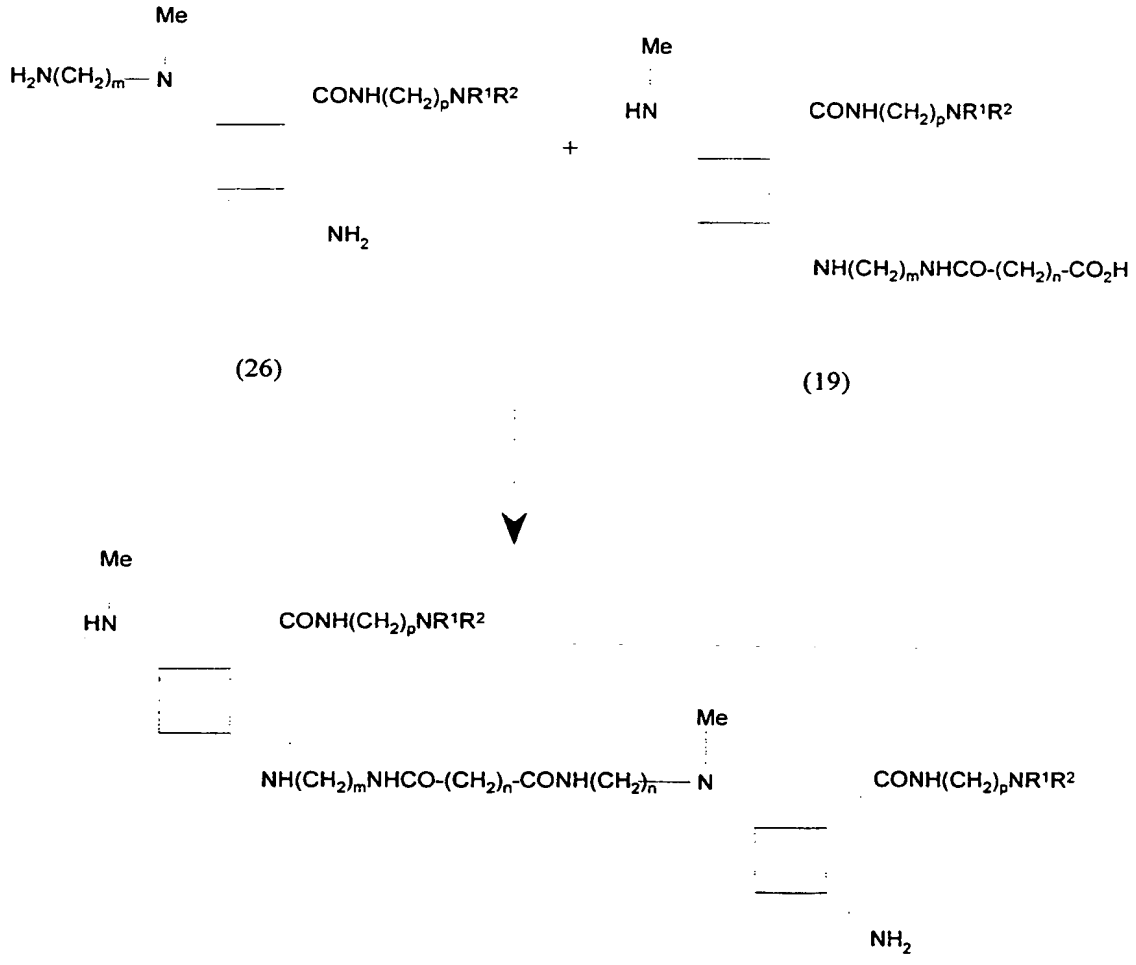
Formula I

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 10, the compound of formula (26) is reacted with a compound of formula (23) in a typical coupling reaction as shown above, for example in Reaction Scheme 6, to give a compound of Formula I [C-N].

Compounds of Formula I wherein the linkage is [N-V] may be prepared by reaction of a compound of formula (26) with a compound of formula (19), as shown in Reaction Scheme 11

REACTION SCHEME 11



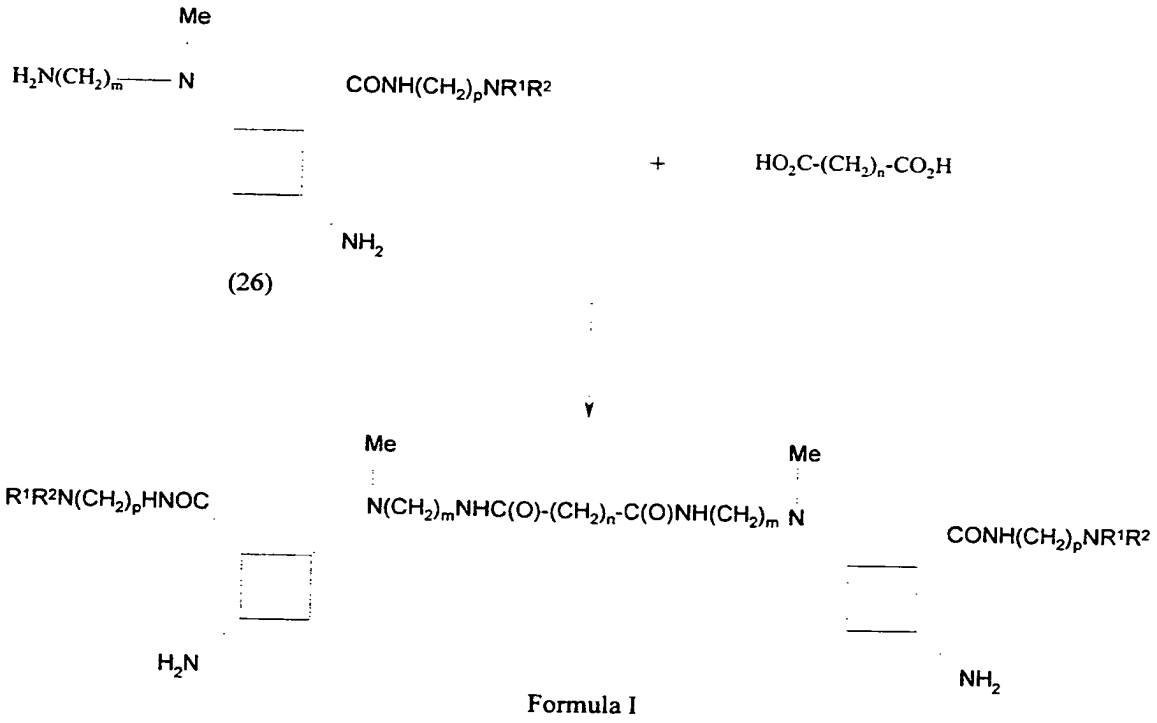
Formula I

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 11, the compound of formula (26) is reacted with a compound of formula (19) in a typical coupling reaction as shown above, to give a compound of Formula I [N-V].

Compounds of Formula I wherein the linkage is [N-N] may be prepared by reaction of a compound of formula (26) with a dicarboxylic acid of formula HO_2C-$(CH_2)_n$-CO_2H, as shown in Reaction Scheme 12.

REACTION SCHEME 12



Formula I

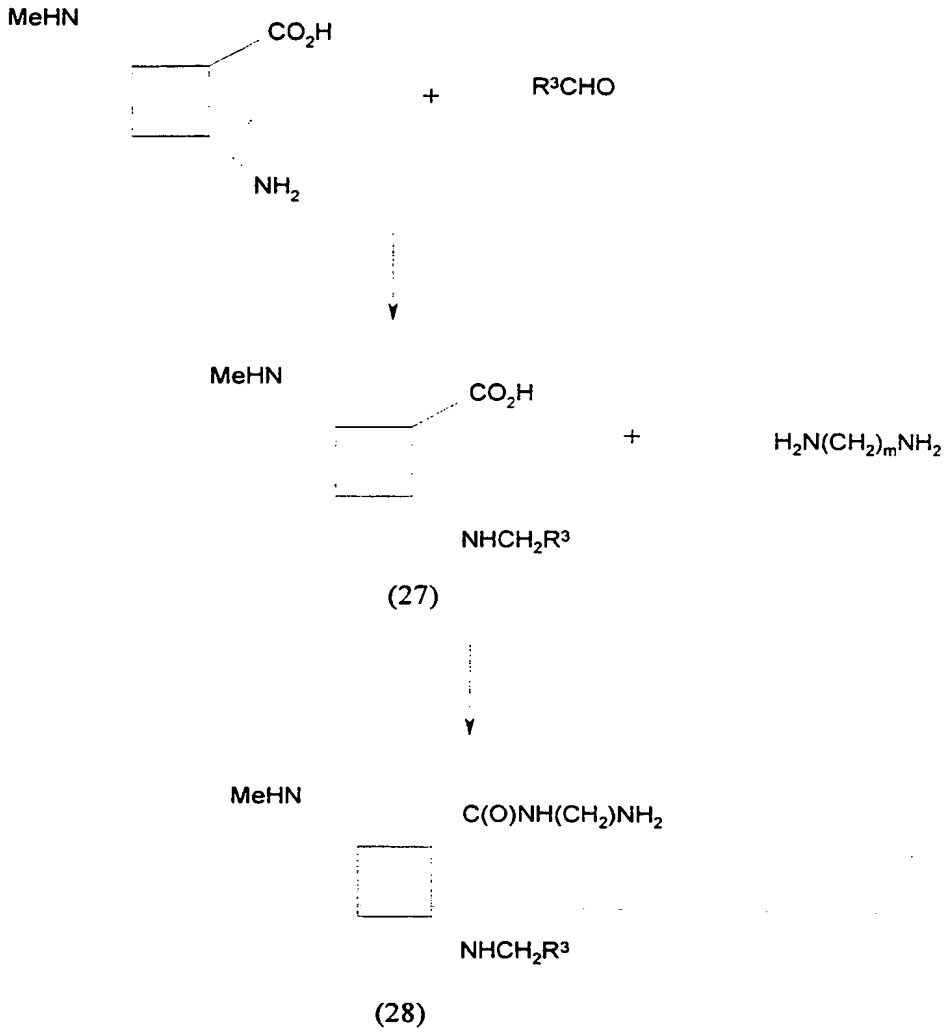
Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 12, the compound of formula (26) is reacted with a dicarboxylic acid of formula HO_2C-$(CH_2)_n$-CO_2H in the same manner as shown above in Reaction Scheme 4, to give a compound of Formula I [N-N].

Other means of linking ligands are shown in Reactions Schemes 13-16 as follows, where the

"box" and the "triangle" represents different ligands.

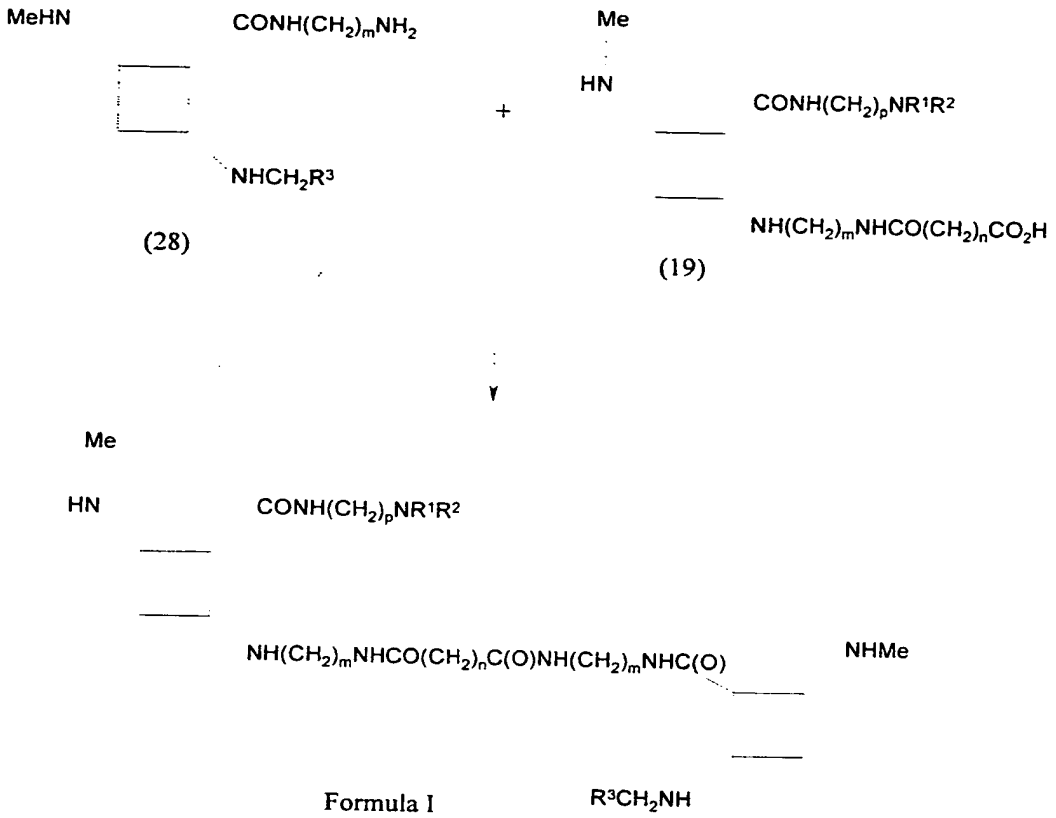
REACTION SCHEME 13



(27)

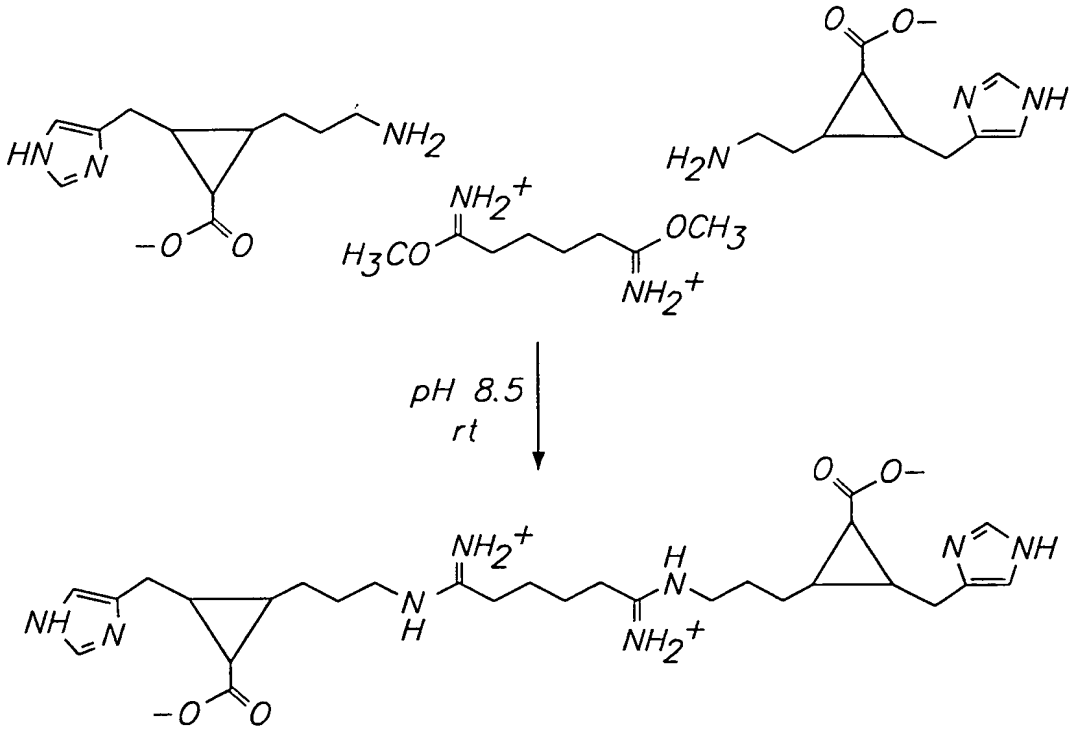
(28)

REACTION SCHEME 14

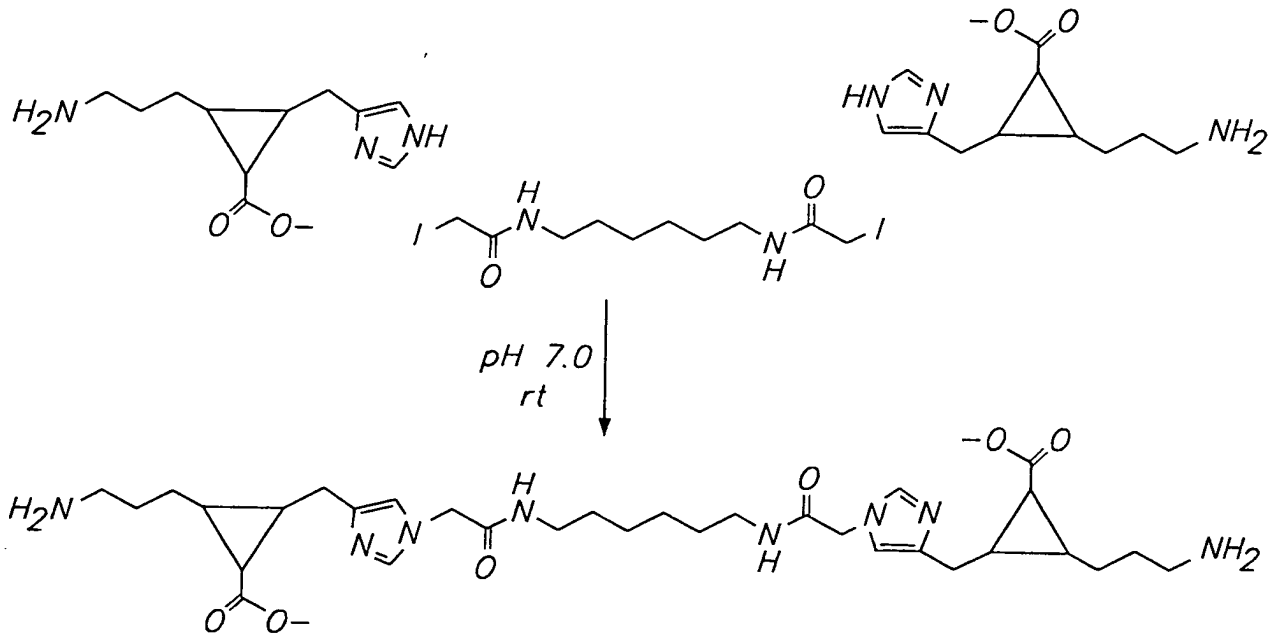


Formula I

REACTION SCHEME 15



REACTION SCHEME 16



Alternative methods of Linking Ligands via Amino Groups

It should be noted that ligands having terminal amino groups may also be linked by reaction with aldehyde linkers (to form a Schiff's base, that can be used as such as a linker or reduced to give a saturated linker). Alternatively, reaction with a disulfonyl halide would give a sulfonamide linker. Another alternative synthesis encompasses reaction of an amine with a diiminoester, which would afford a bis(amidine) linker.

Linking Ligands via Hydroxy Groups

Ligands that include a free hydroxy group in their structure (an alcohol or phenolic hydroxy) may be connected using those hydroxy groups as linkage points by means well known in the art. For example, one synthetic strategy that could be used for linking ligands with free hydroxy groups involves treating the ligand with t-butyl bromoacetate in the presence of a base (e.g. sodium hydride, potassium carbonate) to convert the -OH group to an -O-CH_2CO_2-t-But group, which can be hydrolyzed to an O-CH_2CO_2H group using trifluoroacetic acid. The oxyacetic group can then be used as the linking point for two ligands by making use of the linking strategies shown above for carboxylic acids. For example, reaction of two molar equivalents of the ligand with a diamine of the formula

H_2N-$(CH_2)_n$-NH_2, where n is an integer of 1-20;

gives two ligands connected by a linker of the formula -CH_2CONH-$(CH_2)_n$-$NHCOCH_2$.

Alternatively, treating the hydroxy-bearing ligand with BOC-$NHCH_2CH_2Br$ in the presence of a base (e.g. potassium carbonate) converts the -OH group to an -O-CH_2CH_2NHBOC group, which can be deprotected to give an O-$CH_2CH_2NH_2$ group using trifluoroacetic acid. The oxyethylamino group can then be used as the linking point for two ligands by making use of the linking strategies shown above for amines. For example, reaction of two molar equivalents of the ligand with a dicarboxylic acid of the formula HO_2C-$(CH_2)_n$-CO_2H where n is an integer of 1-20, leads to two ligands being connected by a linker of the formula –CH_2CH_2NHCO-$(CH_2)_n$-$CONHCH_2CH_2$-. Tetracycline and glycopeptide aglycones are examples of ligands that include hydroxy groups suitable for this linking strategy.

Alternatively, converting the hydroxy-group to a leaving group, for example by treatment with mesyl chloride or tosyl chloride, or converting the hydroxy group to a halide by means well known in the art, the ligand can then be linked directly by reaction with a diamine.

The Mannich reaction can be used to link ligands that have an "active" hydrogen in their

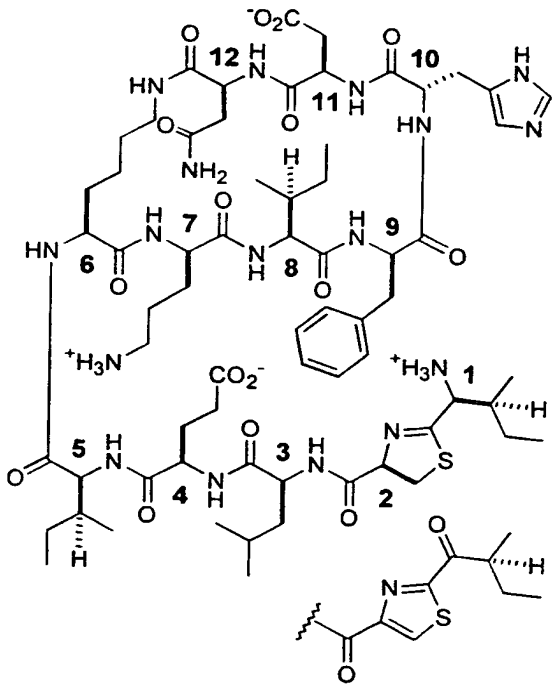
molecular structure. Examples of such active hydrogens are hydrogens that are adjacent to an electron withdrawing group such as a ketone, aldehyde, acid or ester, nitrile, and nitro group, and the like. The Mannich reaction is well known to those skilled in the art, and many reviews in the chemical literature and text books on the Mannich reaction are available. Of particular value is that a linker could be constructed on a ligand having an aromatic moiety, providing there is an active hydrogen present.

Linking Ligands via Amino and Carboxyl Groups

Bacitracin is an antibacterial antibiotic that inhibits an enzymatic process involved in bacterial cell wall biosynthesis. Bacitracin is a widely utilized animal growth promoter and an important component of topical antibacterial ointments. Bacitracin is effective against a subset of both Gram-positive and Gram-negative bacteria in vitro, and it has recently shown promising activity against vancomycin-resistant *Enterococcus faecium* in vitro, and in human studies activity has been shown of enteric eradication upon oral dosing.

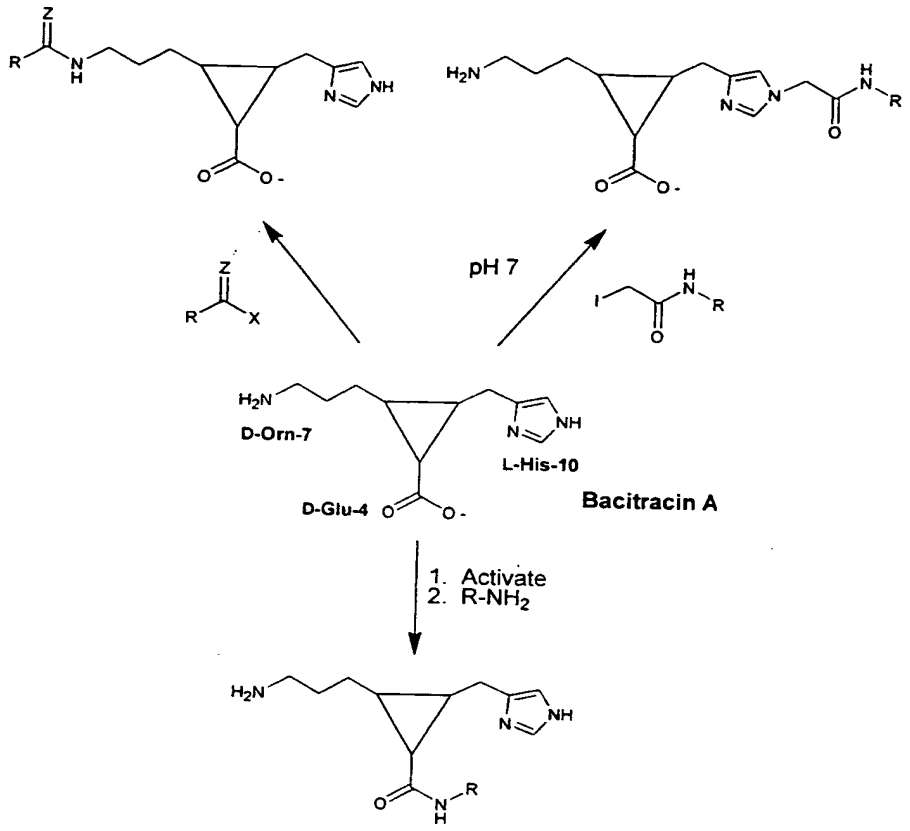
Bacitracin has the following structure:

Figure 1.



Multivalent compounds may be prepared by links through the D-Glu-4, D-Orn-7, and L-His-10 side chains using homo- and heterofunctional framework reagents bearing groups that can be induced to react selectively with carboxyl, amine, and imidazole functional groups. For example, the side chain carboxyl of D-Glu-4 is activated with a carbodiimide, phosphoryl, or uronium ion reagent which then reacts with an amino group of a linker to form a stable amide linkage. These reactions are typically carried out in polar organic solvents such as dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO); however, they may also be carried out under buffered aqueous conditions. The side chain amino group of D-Orn-7 reacts with activated acyl groups (e.g., N-hydroxysuccinimide esters, imido esters) to form stable amide or amidinium linkages. In the case of reaction with the imido thioester 2-iminothiolane, acylation of the D-Orn-7 side chain results in production of a free thiol group that may subsequently be coupled selectively with a maleimide group or alpha-acetamide group of the linker. These reactions may be carried out in either aqueous buffers or in organic solvents such as DMF or DMSO. The side chain imidazole group of L-His-10 reacts selectively with iodoacetamide groups on the framework to form alkylimidazole linkages. Examples of these chemistries are presented below, where the triangle represents the core of the bacitracin molecule.

Figure 2.



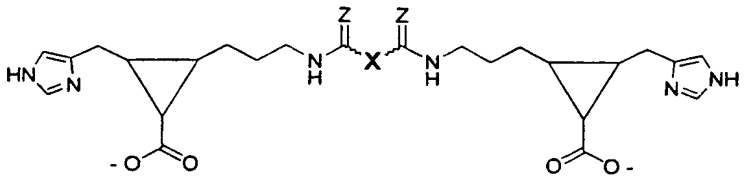
where R is a linking moiety.

It should be noted that any ligand having similar groups could be linked via this procedure. The desired products are isolated through standard purification techniques, most typically reversed-phase HPLC.

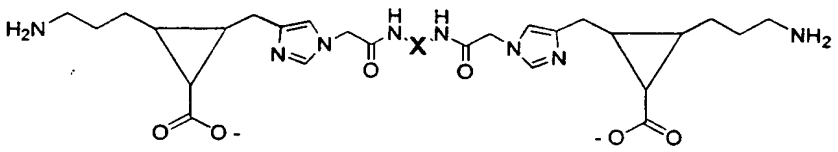
Bacitracin divalomers are prepared in each of the six possible orientations: Orn-Orn, His-His, Glu-Glu, Orn-His, Glu-Orn, His-Glu (Figure 1).

Figure 3.

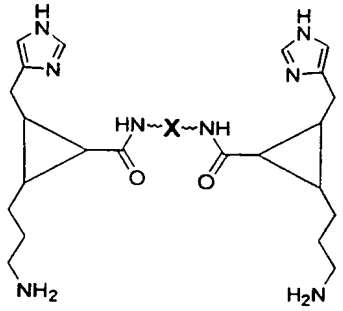
Orn-Orn



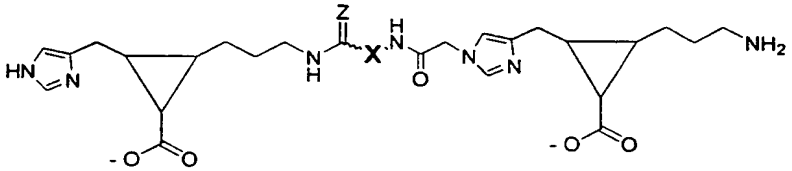
His-His



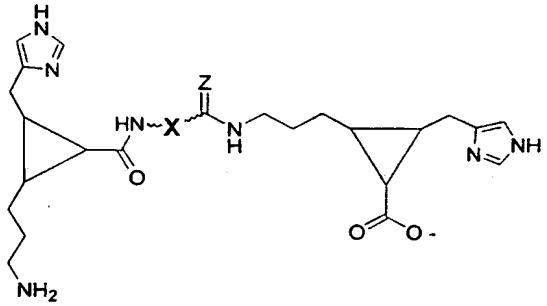
Glu-Glu



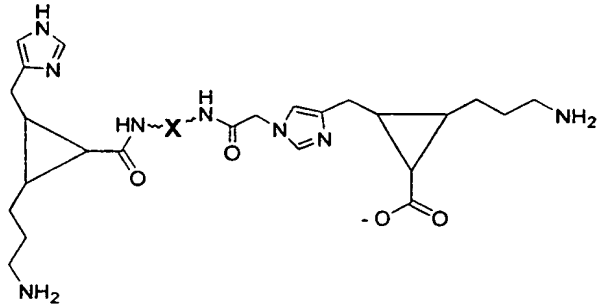
Orn-His



Glu-Orn



Glu-His

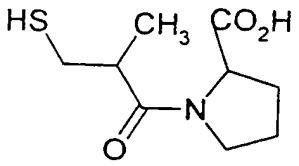


where X is the linker.

In addition to the functional groups necessary to attach to bacitracin subunits, the linkers for this array are preferably peptide, polyether, and hydrocarbon elements. The size of these linkers ranges between 2 and 50 linear heavy atoms separating the two subunits.

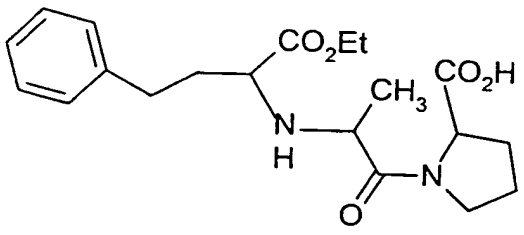
Determining the in vitro antibacterial properties of the compounds in the first-generation array is one manner in which structure-activity relationships may be determined. In particular, these results indicate which relative orientations of bacitracin subunits, which linker sizes, and which linker compositions afford the highest levels of activity. This information is then used in the design of a second-generation array in which the influence of the degree of multivalency is probed. Here, a series of bacitracin trivalomers and tetravalomers are prepared in linear, radial, and three-dimensionally disposed geometries using the most promising relative subunit orientations, inter-subunit spacings, and linker compositions as indicated by the results with the first-generation array.

Captopril is a ligand that is an ACE inhibitor, useful as an antihypertensive, and has the following structure.



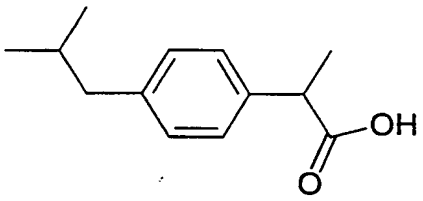
There are many sites that are appropriate for use in linking multiple copies of captopril together. For example, captopril can be linked via the carboxyl group using the procedures detailed above. The ligand may also be linked via attachment to the proline ring, the methyl group, the thiol group, etc., as shown.

Another example of an ACE inhibitor is enalapril, which has the following structure.



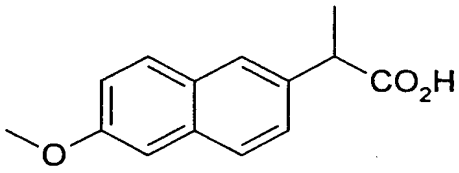
Enalapril can be linked in the same manner as captopril.

Ibuprofen is a ligand that has antiinflammatory and analgesic activity; it has the structure:



As for most ligands, there are several sites suitable for use in linking multiple copies of ibuprofen together. For example, ibuprofen can be linked via the carboxyl group using the procedures detailed above. Alternatively, ibuprofen could be linked via the alpha methyl group, for example by starting with the corresponding-acetic acid derivative and linking at the alpha position using a dihalide and a strong base.

Naproxen is another ligand that has antiinflammatory and analgesic activity; it has the following structure:



As for ibuprofen, sites suitable for linking include the carboxyl group, the alpha methyl group, the naphthyl group, etc.. Additionally, the 6-methoxy position could be utilized for linking by, for example, starting from 6-hydroxynaphthyl-2-propionic acid (protected as an ester), and linking the hydroxy groups as detailed above.

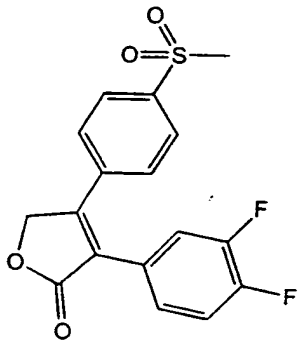
Inhibitors of Cyclooxygenase-2 (COX-2)

Inhibitors of cyclooxygenase are useful as anti-inflammatory agents. They are indicated for a variety of common aches and pains, as well as more severe forms of inflammation such as rheumatoid arthritis. The most common side effects are gastrointestinal disorders, especially ulcerative lesions and bleeding. Decreased renal function isobserved in some patients.

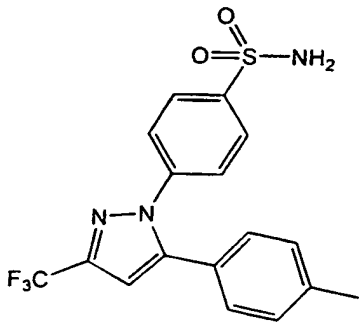
It has been discovered that there are two isoforms of the enzyme, designated COX-1 and COX-2. Inhibition of COX-1 gives rise to the undesirable side effects observed with NSAIDs,

and consequently selective inhibitors of COX-2 provide advantages over current therapies.

Two known COX-2 inhibitors have the following structures:

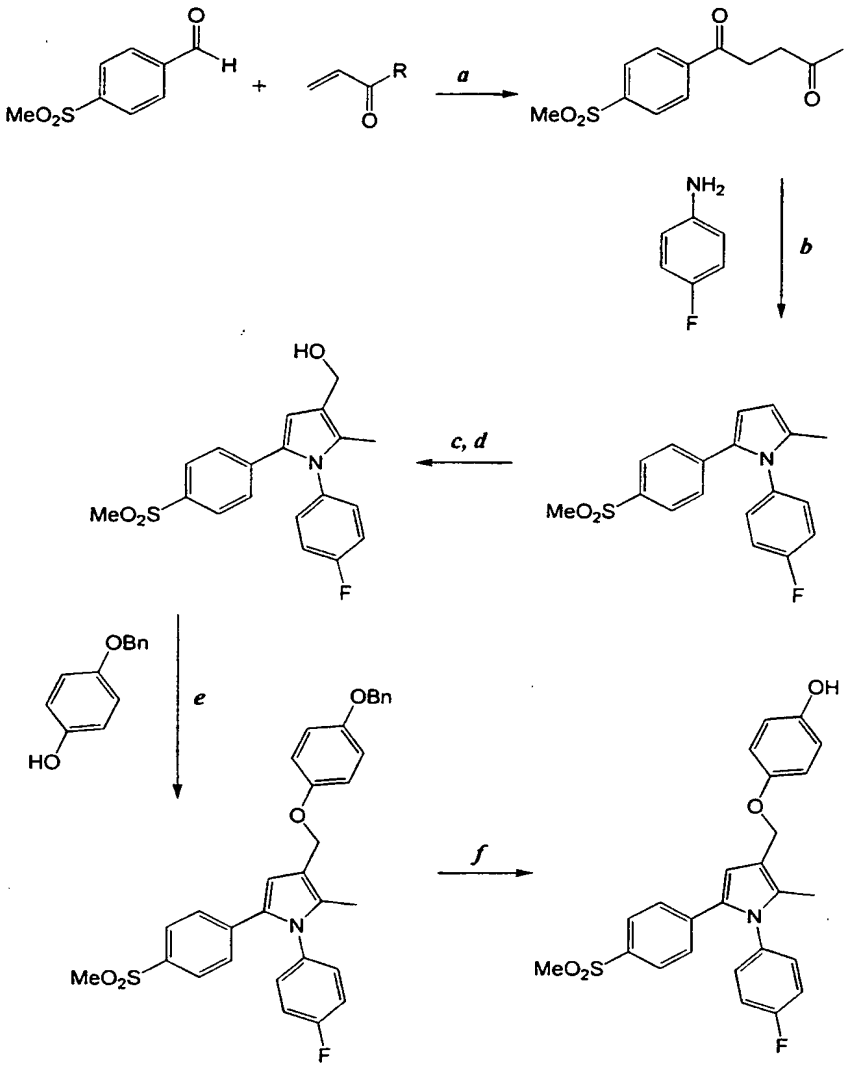


MK-966



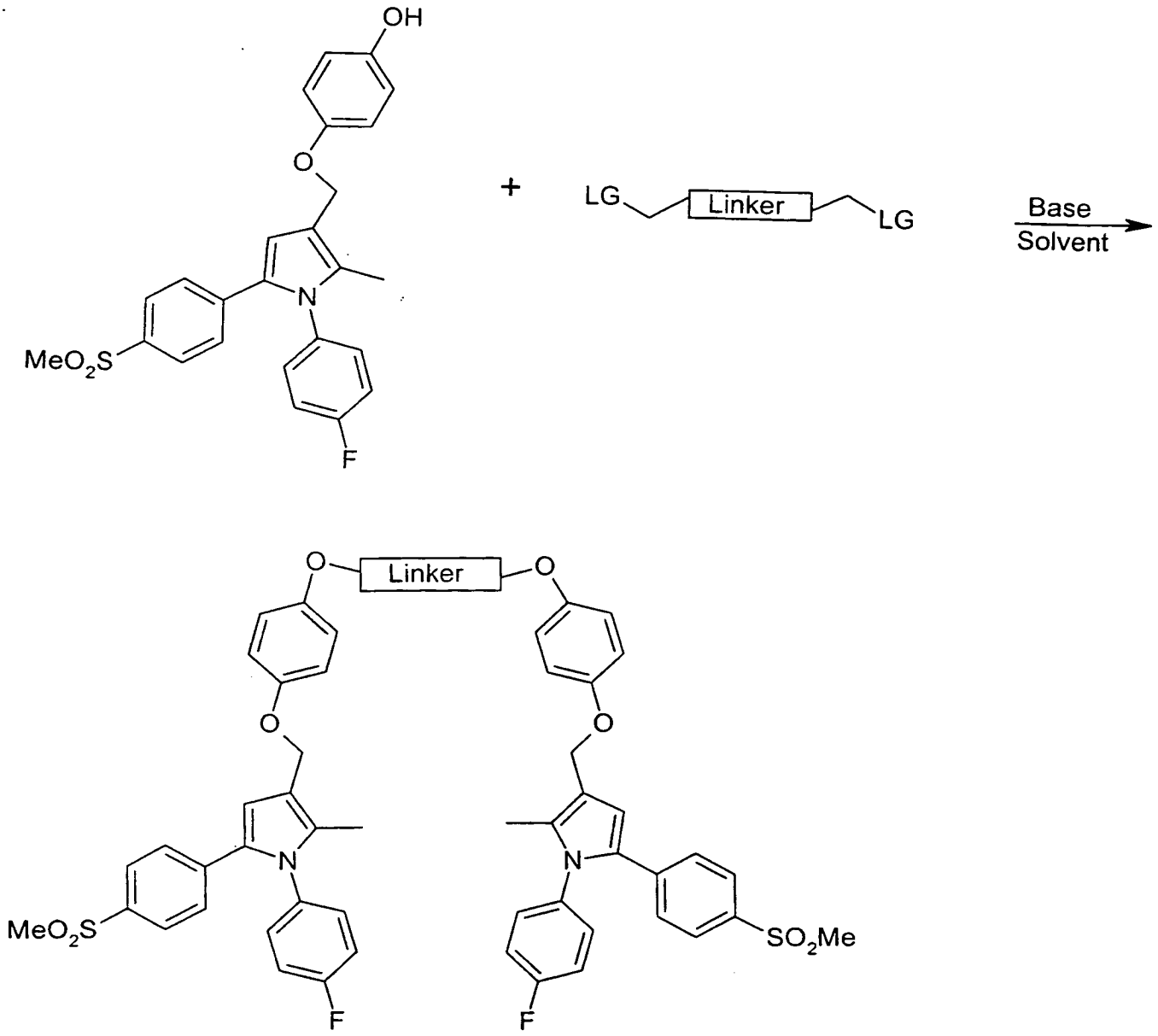
SC-58635

These structures are representative of known COX-2 inhibitors. Accordingly, these ligands and those of similar structure may be dimerized using those techniques shown below as an example, i.e. introducing a 4-hydroxyphenoxymethyl group on the aromatic ring and using that as a "handle" for linking as shown above for hydroxy groups.



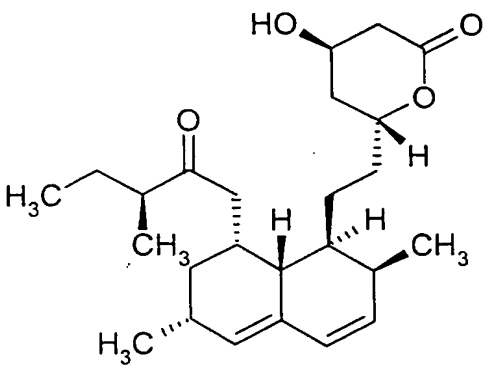
Reagents and conditions. a) Et_3N, EtOH, thiazolium, reflux 5 h. b) Toluene, TsOH, reflux 20 h. c) DMF, $POCl_3$. d) $NaBH_4$, EtOH. e) DEAD, PPh_3, RT 48 h. f) H_2, Pd/C, EtOH

5



Where
"base" is an organic or inorganic base capable of deprotonating the phenolic moiety under the chosen conditions; examples include but are not limited to Na2CO3, K2C03, Cs2CO3, Et3N, iPr2EtN, NaOH, KOH, NaOEt, NaH, etc...
"solvent" is chosen from the list of defined solvents, including but not limited to THF, DMF, acetone, water, DMA, NMP, 2-butanone, etc...
and "LG" is an appropriate leaving group, including but not limited to iodide, bromide, chloride, mesylate, tosylate, etc...

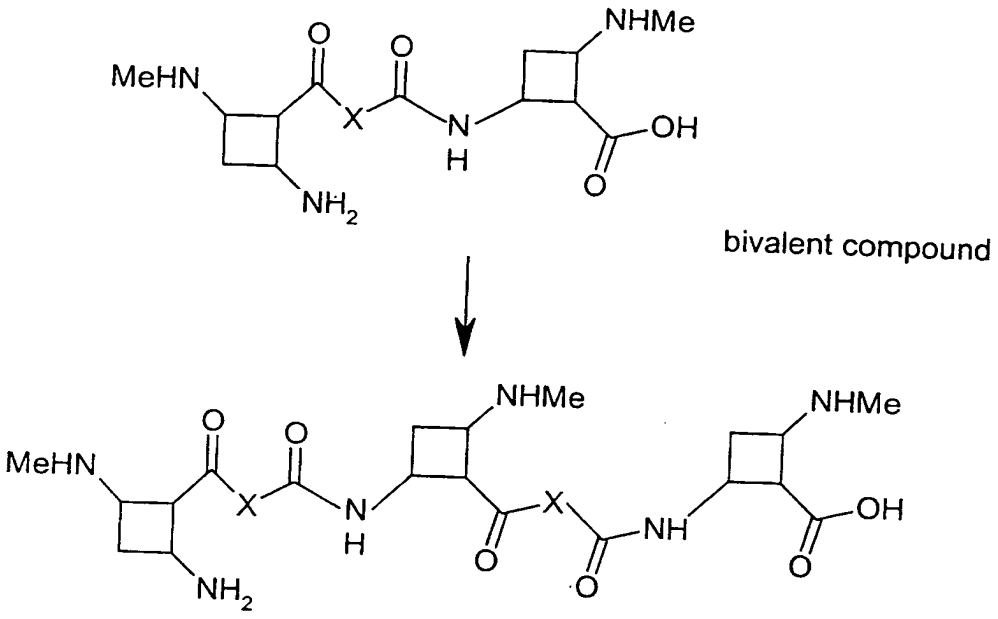
HMG-CoA Reductase is the rate controlling enzyme in cholesterol biosynthesis. Consequently, drugs such as the "statins" that inhibit this enzyme are useful for lowering cholesterol levels. One example is lovastatin, which has the structure:



As previously mentioned, the preferred compounds of Formula I are bivalent compounds, and their preparation is shown in detail above. Compounds of Formula I wherein p is greater than 2 (i.e. 3-10) are prepared in a manner analogous to bivalent compounds, as described below.

Compounds of Higher Valency

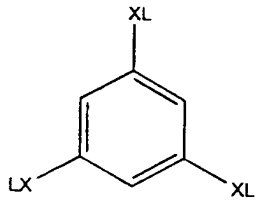
Trivalent compounds may be prepared from bivalent compounds, for example by reacting the free carboxyl group of a bivalent compound of Formula I as prepared in Reaction Scheme 8 [C-N] with an appropriate linker, which is in turn reacted with a ligand, thus giving three ligands linked together (i.e. of the type L-X-L-X-L). Alternatively, the bivalent compound of Formula I as prepared in Reaction Scheme II could be reacted further with an appropriate linker and ligand to give a trivalent compound.



Trivalent compound, where- X-C(O)- is a linker

Clearly, other compounds of Formula I may be similarly prepared as multibinding agents, i.e. tetravalent, pentavalent, hexavalent, heptavalent, octavalent, nonavalent, and decavalent compounds.

Alternatively, reaction of three molecules of a ligand bearing a carboxyl group with one molecule of a cyclic linker, such as 1,3,5-tris-(aminomethyl)benzene, yields a trimeric compound that is potentially trivalent. Other cyclic oligomers can be similarly prepared.

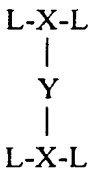


Where X represents $-CH_2NH-$; and

L is a ligand.

Tetravalent compounds may be prepared as shown above. An alternative configuration for a

tetravalent compound would be obtained by dimerizing a bivalent compound by connecting two molecules of a bivalent compound via its linker. This could be represented as follows:



Where L is a ligand that is the same or different at each occurrence;
X is a linker; and
Y is a linker that is the same as X or different.

Isolation and Purification of the Compounds

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the Examples hereinbelow. However, other equivalent separation or isolation procedures could, of course, also be used.

In the following examples:
Examples 1-7 provide a description of the syntheses disclosed in Reaction Schemes 1-16.
Examples:8-11 demonstrate linking at various positions of ligands that bind to the Microsomal Triglyceride Transferase Protein;
Examples 12-21 demonstrate linking at various positions of ligands that bind to Reverse Transcriptase.
Examples 22-23 demonstrate linking at various positions of ligands that bind to HMG CoA Reductase;
Examples 24-27 demonstrate linking at various positions of ligands that bind to Cyclooxygenase;
Examples 28-32 demonstrate linking at various positions of ligands that bind to Nitric Oxide Synthase;

Examples 33-38 demonstrate linking at various positions of ligands that bind to Phosphodiesterase;

Examples 39-44 demonstrate linking at various positions of ligands that bind to Topoisomerase; and

Examples 45-48 demonstrate linking at various positions of ligands that bind to DNA Gyrase.

The linking points of the individual ligands are indicated by arrows.

EXAMPLE 1

Preparation of [C-C] Compounds of Formula I

(1) Preparation of a Compound of Formula (3) in which m is 2 and n is 3

To a solution of tert-butyl *N*-(2-aminoethyl)carbamate (2.3g, 14.4mmol) and *N,N*-diisopropylethylamine (2.5ml, 14.3mmol) in 15mL methylene chloride at O°C is added glutaryl dichloride (0.6mL, 4.7mmol) in 15mL methylene chloride dropwise. The resulting mixture is allowed to warm to room temperature with stirring while adding water (15mL). The methylene chloride is removed under reduced pressure and more water is added (30mL). The resulting suspension is filtered and washed sequentially with 10% potassium hydrogen sulfate, water, saturated sodium bicarbonate, and water. The solid is dried under vacuum yielding 1.3g (3.1 mmol, 66%) of pentanedioic acid bis-[(2-t-butoxycarbonylaminoethyl)amide], a compound of formula (3).

Similarly, varying the composition of m and n, other compounds of formula (3) are prepared.

(2) Preparation of a Compound of Formula (4) in which m is 2 and n is 3

Pentanedioic acid bis-[(2-t-butoxycarbonylaminoethyl)amide], a compound of formula (3) (1.3g, 3.1 mmol) is suspended in 15 mL methylene chloride. 15 mL of trifluoroacetic acid is added at room temperature giving (with effervescence) a solution that is stirred for 40 minutes, then evaporated *in vacuo*. The residue is dissolved in methanol and treated with 3mL 4N hydrogen chloride in dioxane followed by diethyl ether, giving a gum. The liquids were decanted and the gum dried under vacuum yielding 1.0g (3.4mmol) of pentanedioic acid bis-[(2-aminoethyl)amide], a compound of formula (4).

Similarly, varying m and n, other compounds of formula (4) are prepared.

(3) Preparation of a Compound of Formula I

At room temperature, a ligand bearing an amino group (2.3mmol) is dissolved in 36mL of dimethylsulfoxide. To this solution is added pentanedioic acid bis-[(2-aminoethyl)amide], a compound of formula (4) (1.0g, 3.4mmol suspended in 27mL N,N-dimethylformamide) followed by

compound of formula (4) (1.0g, 3.4mmol suspended in 27mL N,N-dimethylformamide) followed by N,N-diisopropylethylamine (2.4mL, 13.8mmol). The resulting suspension is stirred at room temperature for several hours until it is mostly soluble. Then a solution of PyBOP (1.3g, 2.5 mmol) and 1-hydroxybenzotriazole (310mg, 2.3mmol) in 9mL N,N-dimethylformamide is added rapidly dropwise. The mixture is stirred at room temperature for 1 hour and then added dropwise to 600mL of acetonitrile, giving a precipitate that is filtered, washed with acetonitrile ,then diethyl ether, and dried under vacuum. The crude product is purified by reverse phase HPLC (50 minute 2-30% acetonitrile in water containing 0.1% trifluoroacetic acid to yield a compound of formula (5) contaminated with1-hydroxybenzotriazole and a compound of Formula I as their respective trifluoroacetic acid salts.

(4) Preparation of other Compounds of Formula I

Similarly, following the procedures of Example 1, steps 1-3, other compounds of Formula I are prepared.

EXAMPLE 2

Alternative Preparation of [C-C] Compounds of Formula I

(1) Preparation of a Compound of Formula (7) in which m is 2

At room temperature an amino bearing ligand (7.3g, 4.7mmol) is dissolved in 75mL of dimethylsulfoxide. To this solution is added *N,N*-diisopropylethylamine (4.1mL, 23.5mmol) followed by 9-fluorenylmethyl *N*-(2-aminoethyl)carbamate hydrochloride (1.8g, 5.6mmol). To the resulting solution at room temperature is added rapidly dropwise a solution of PyBOP (2.7g, 5.2mmol) and 1-hydroxybenzotriazole (630mg, 4.7mmol) in 75mL 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone. The resulting solution is stirred at room temperature for 2 hours, then poured into 800mL diethyl ether, giving a gum. The diethyl ether is decanted and the gum washed with additional diethyl ether to give a compound of formula (7).

(2) Preparation of a Compound of Formula (8) in which m is 2

The gum of formula (7) is then taken up in 40mL of *N,N*-dimethylformamide, to which 10mL of piperidine is added and the solution left to stand at room temperature for 20 minutes. The solution is then added dropwise to 450mL of acetonitrile giving a precipitate. Centrifugation is followed by decantation of the acetonitrile and the residue wahed twice with 450mL of acetonitrile, once with 450mL of diethyl ether and air dried. The residue is taken up in water, acidified to pH<5 with a

small amount of 3N hydrochloric acid and purified by reverse-phase HPLC using a gradient of 2-30% acetonitrile in water containing 0.1% trifluoroacetic acid yielding a compound of formula (8).

(3) Preparation of a Compound of Formula I

Compound (8) (400mg, 220μmol) and glutaric acid (10mg, 76μmol) were dissolved in 5mL *N,N*-dimethylformamide and *N,N*-diisopropylethylamine (140μL, 800μmol) followed by PyBOP (83mg, 160μmol) and 1-hydroxybenzotriazole (10mg, 74μmol) in 500μL *N,N*-dimethylformamide. The reaction is stirred for 75 minutes at room temperature then an additional 20mg of PyBOP is added. 75 minutes later the solution is dripped into 45mL acetonitrile. The resulting precipitate is collected by centrifugation, washed with ether, air dried and purified by reverse-phase HPLC (50 min 2-30% acetonitrile in water containing 0.1%trifluoroacetic acid, elutes at 33 min) to give a compound of Formula I (AMI 265) as its trifluoroacetic acid salt.

(4) Preparation of other Compounds of Formula I

Accordingly, following the procedures of Example 2, steps 1-3, other [C-C] compounds of Formula I according to the table below were prepared.

EXAMPLE 3

Preparation of a [C-V] Compound of Formula I in which Position C' is Substituted

(1) Preparation of a Compound of Formula (22) in which m and n are both 2

To a solution of an amino-bearing ligand (26.8 μmol) in DMF (2.0 mL) is added a compound of the formula:

$HO_2CCH_2CH_2NHOCCH_2CH_2NHOCCH_2CH_2CH_2CONHCH_2CH_2CONHCH_2CH_2CO_2CH_2FM$

(FM refers to 9-Fluorenyl) (20.0 mg, 26.8 μmoll), followed by PyBOP (20.9 mg, 40.2 μmol), HOBt (5.40 mg, 40.2 μmol), and Hunig's base (23.3 μL, 134 μmol). The reaction solution is stirred for 1 hour and then added dropwise to 20mL of acetonitrile giving a precipitate, which is collected by centrifugation. The crude precipitate is dried in air, yielding a compound of formula (22): MS calculated: MH^+, 2068; Found, 2068. The compound is used in the next step without further purification.

(2) Preparation of a Compound of Formula (23) in which m and n are both 2

The compound of formula (22) is dissolved in 1mL of DMF, and 100μL of piperidine added to the solution. The solution is allowed to stand at room temperature for 30 minutes,

following the course of the reaction by mass spectroscopy. The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes). The desired product is identified by mass spectroscopy using an API 300 electrospray mass spectrometer and afterwards lyophilized to a white powder to afford compound (23) as a white powder. MS calculated: MH^+, 1890; Found, 1890.

(3) Preparation of a Compound of Formula I

The compound of formula (23) prepared above (4.80 μmol) is dissolved in 500 μL of DMF. A compound of formula (17) (4.80 μmol) is added to the solution, followed by PyBOP (2.50 mg, 4.8umol), HOBt (0.65 mg, 4.80 μmol) and Hunig's base (6.70 μl, 38.4 μmol). The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes). The desired product is identified by mass spectroscopy using an API 300 electrospray mass spectrometer

EXAMPLE 4
Preparation of a [C-N] Compound of Formula I

(1) Preparation of a Compound of Formula (24) in which m is 2

An amin- bearing ligand (2.60mmol) is suspended in 40 mL of 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone and heated to 70^0C for 15 minutes. N-(9-fluorenylmethoxycarbonyl)-aminoacetaldehyde (720 mg, 2.6 mmol) is added and the mixture is heated at 70^0C for one hour. Sodium cyanoborohydride (160 mg, 2.5 mmol) in 2 mL methanol is added and the mixture is heated at 70^0C for 2 hours, then cooled to room temperature.). The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water

as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 10-70% B over 90 minutes), which yields a compound of formula (24) as its trifluroacetate salt.

(2) Preparation of a Compound of Formula (25) in which m is 2 and p is 3

The compound of formula (24) obtained above (150 μmol) is dissolved in 3mL of DMF. 3-(dimethylamino)propylamine (28.3 μL, 225 μmol) is added, followed by the addition of PyBOP (85.8 mg, 165 μmol), HOBt (20.3 mg, 150 μmol) and Hunig's base (65.0 μL, 375 μmol). The reaction solution is stirred for 1 hour and then added dropwise to 20mL of acetonitrile giving a precipitate, which is collected by centrifugation, to give compound of formula (25) as a white solid.

(3) Preparation of a Compound of Formula (26) in which m is 2 and p is 3

The compound of formula (25) obtained above is dissolved in 1mL of DMF, and 100 μL of piperidine added to the solution. The solution is allowed to stand at room temperature for 30 minutes and the course of the reaction is followed by mass spectroscopy. The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yields the compound of formula (26) as its trifluroacetate salt.

(4) Preparation of a Compound of Formula I

The compound of formula (26) prepared above (3.14 μmol) is dissolved in 500 μL of DMF. The compound of formula (19) (3.14 μmol) is added to the solution, followed by PyBOP (2.44 mg, 4.8 μmol), HOBt(0.65 mg, 4.8 μmol) and Hunig's base (6.7 uL, 38.4 μmol).). The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yielded a compound of Formula I as its trifluroacetate salt.

EXAMPLE 5
Preparation of an [N-N] Compound of Formula I

(1) Preparation of a Compound of Formula I

The compound of formula (26) prepared above (12.7 μmol) is dissolved in 500μL of DMF, and a compound of the formula:

$HO_2CCH_2CH_2NHOCCH_2CH_2NHOCCH_2CH_2CH_2CONHCH_2CH_2CONHCH_2CH_2CO_2H$

(2.64 mg, 6.34 μmol) is added, followed by PyBOP (8.24 mg, 15.8 μmol), HOBt(2.13 mg, 15.8 μmol) and Hunig's base (8.8 μl, 51.0 μmol). The reaction solution as added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yields a compound of formula I as its trifluroacetate salt.

EXAMPLE 6
Preparation of a [C-V] Compound of Formula I

(1) Preparation of a Compound of Formula (27) in which m is 2

An amino-bearing ligand (3.2 mmol) is suspended in 40mL of 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone and heated to 70°C for 15 minutes. 4-Butoxybenzaldehyde (570 mg, 3.2 mmol) is added and the mixture heated at 70°C for 1hour. Sodium cyanoborohydride (241 mg, 3.8 mmol) in 2 mL methanol is added and the mixture heated at 70°C for 2 hours, then cooled to room temperature. The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 10-70% B over 90 minutes), yields a compound of formula (27) as its trifluroacetate salt.

2. Preparation of a compound of Formula (28) where m is 2

The compound of formula (27) prepared above (45.0 μmol) is dissolved in 2mL of DMF. Ethylene diamine (13.4 mg, 22.3 μmol) is added followed by PyBOP (28.0 mg, 54.0 μmol),

HOBt (7.2 mg, 54.0 μmol) and Hunig's base (63.0 μL, 360 μmol). The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), yields a compound of formula (28) as its trifluroacetate salt.

3. Preparation of a [C-V] compound of Formula I in which one V position is substituted, wherein m and n are2 and p is 3

The compound of formula (28) prepared above (4.9 μmol) is dissolved in 500 μL of DMF. The compound of formula (19) (10.0 mg, 4.9 μmol) is added to the solution, followed by PyBOP (3.06 mg, 5.9 μmol), HOBt (0.80 mg, 5.9 μmol) and Hunig's base (6.7 uL, 38.4 μmol). The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), yielding a compound of Formula I as its trifluroacetate salt

EXAMPLE 7

Preparation of a Bacitracin Bivalent Compounds of Formula I

(1) Synthesis of bis(Orn,Orn-bacitracin)-1,6-hexanediamidine

A solution of bacitracin A (1.0 mmol, 1.42 g) in 100 mL 0.1 M N-ethylmorpholineacetic acid buffer, pH 8.5 is treated with solid dimethyl adipimidate (0.50 mmol, 0.12 g). The resulting mixture is stirred at room temperature for 2 hours, and then fractionated by reversed-phase HPLC using a gradient of 0.1% trifluoroacetic acid(TFA)/acetonitrile in 0.1% aqueous TFA to yield the symmetrical Ornithine-modified dimer.

(2) Synthesis of bis(His,His-bacitracin)-1,6-hexanediacetamide

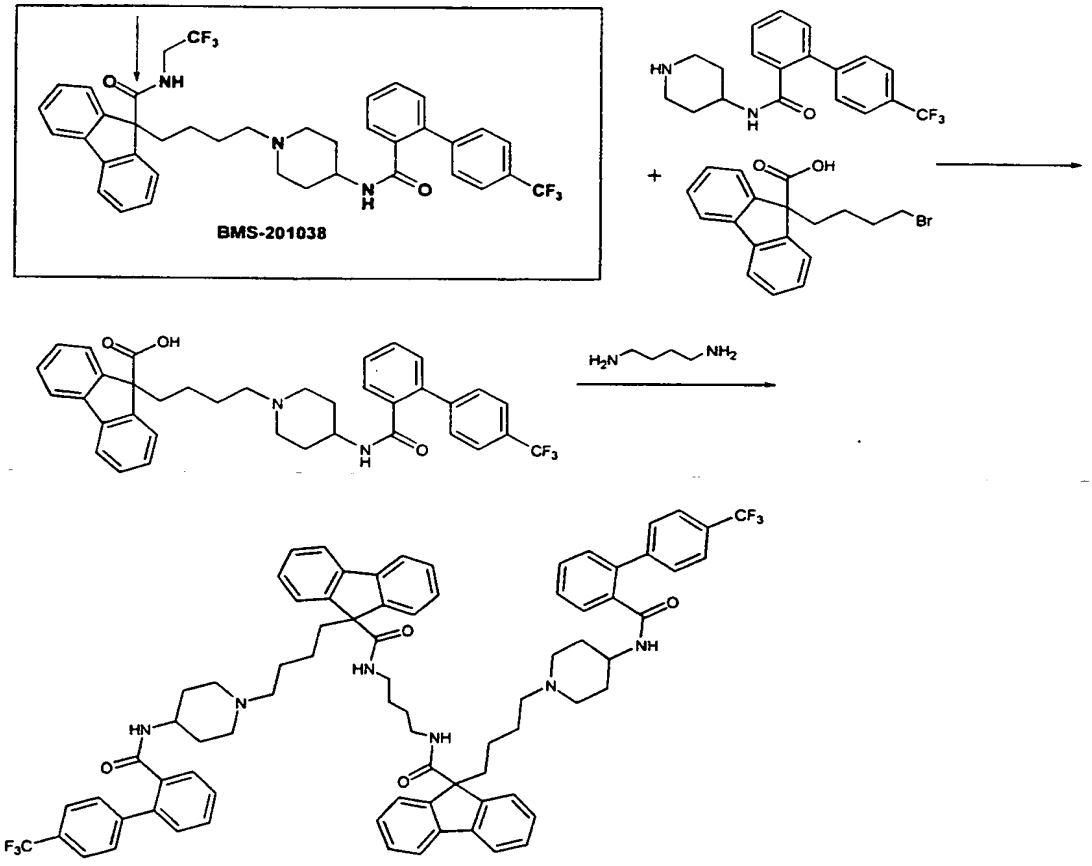
A solution of bacitracin A (1.0 mmol, 1.42 g) in 100 mL phosphate buffered saline, pH 7.0 is treated with solid 1,6-hexanediiodoacetamide (0.50 mmol, 0.23 g). The resulting mixture is stirred at room temperature for 2 h, and then fractionated by reversed-phase HPLC using a gradient of 0.1% TFA/acetonitrile in 0.1% aqueous TFA to yield the symmetrical Histidine-

modified dimer.

(3) Synthesis of bis(Glu,Glu-bacitracin)1,14-(3,6,9,12-tetraoxa)-tetradecanediamide

A solution of bacitracin A (1.0 mmol, 1.42 g) and 1,14-(3,6,9,12-tetraoxa)-tetradecanediamine (0.5 mmol, 0.12 g) in 100 mL dimethyl formamide is treated sequentially N,N-diisopropylethylamine (0.35 mL, 2.0 mmol), Nhydroxybenzotriazole (1.0 mmol, 0.14 g) and PyBOP (1.0 mmol, 0.52 g). The reaction is then stirred at room temperature for 2 h. The solvent is removed under vacuum. The residue is then redissolved in 0.1% aqueous TFA and fractionated by reversed-phase HPLC using a gradient of 0.1% TFA/acetonitrile in 0.1% aqueous TFA to yield the symmetrical glutamic acid-modified dimer.

EXAMPLE 8

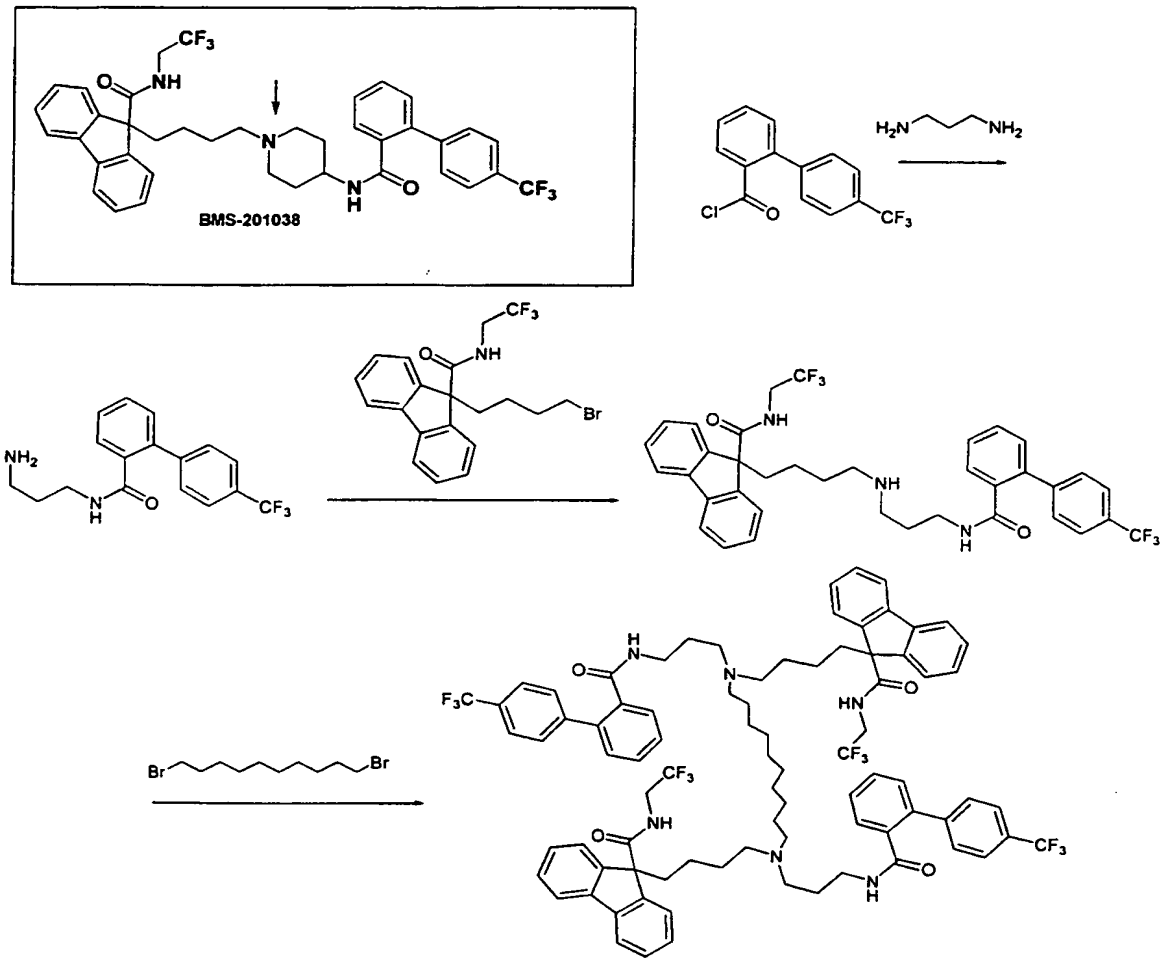


A solution of the starting amine as the hydrochloride (18.0 g, 49 mmol) in 100 mL dimethylformamide is stirred under argon at room temperature and treated with potassium

carbonate (12.6 g, 49 mmol) followed by with [1-bromobutyl]-9H-fluorene-9-carboxylic acid (16.9 g, 49 mmol) (described in US 5,712,279). The reaction is heated to 50 C for 24 h. After cooling, the reaction is filtered to remove potassium carbonate, and the filter cake is rinsed with ethyl acetate. The solvents are removed in vacuo to afford a solid from which the desired product is obtained after recrystallization from ethanol.

The above product (4.0 mmol) is dissolved in 10 mL anhydrous dimethylformamide and treated with hydroxybenzotriazole (5.0 mmol), diisopropylethyl amine (4.0 mmol), PyBOP (4.0 mmol), and 1,4-diaminobutane (2.0 mmol). The coupling reaction is stirred overnight at room temperature. Volatiles are removed under vacuum and the crude product is fractionated by reverse-phase HPLC to afford the desired product after lyopholization of the appropriate fractions.

EXAMPLE 9



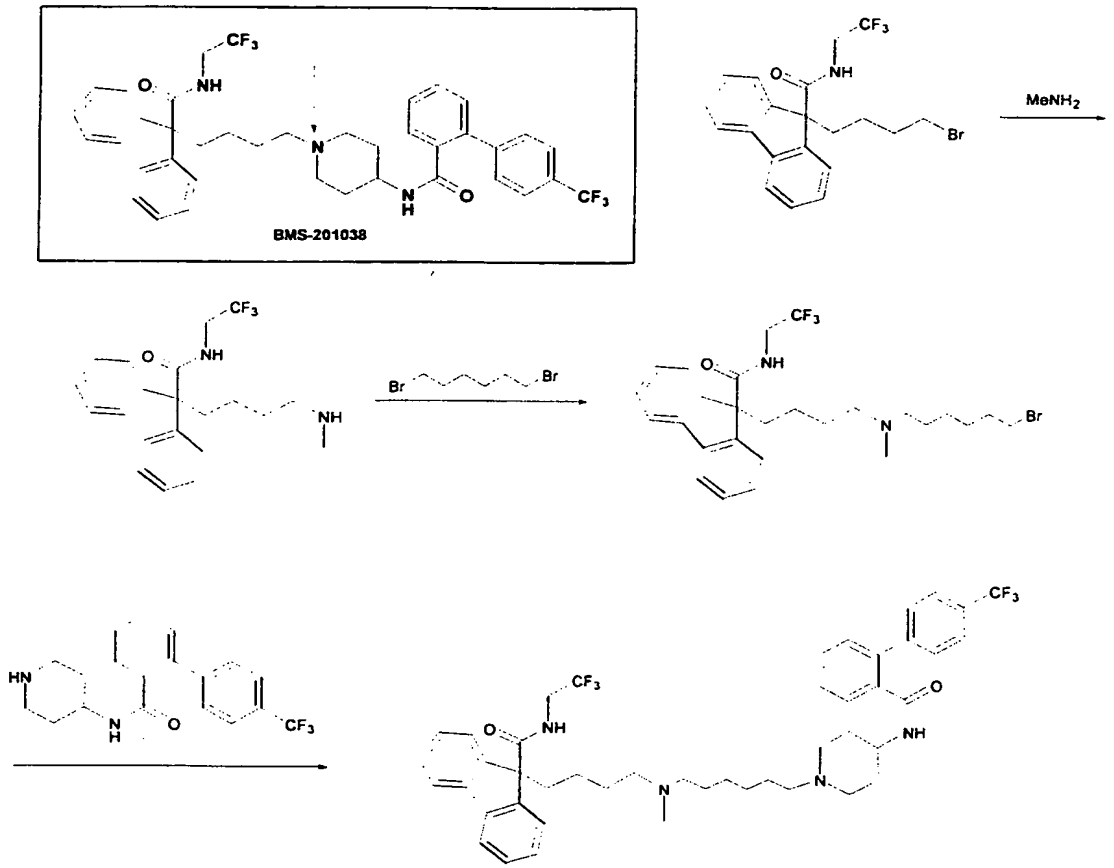
The acid chloride derivative of 4-(trifluoromethyl)-biphenyl-2'-carboxylic acid is generated as described in US 5,712,279. To a slurry of 4-(trifluoromethyl)-biphenyl-2'-carboxylic acid (50.0 g, 190 mmol) in 500 mL methylene chloride is added oxalyl chloride (28.7 mL, 330 mmol) followed by five drops of dimethylformamide. The reaction bubbles vigorously and is stirred at room temperature under argon for 2 h. At this time all solid has dissolved and gas evolution has ceased. The solvent is removed in vacuo, and the residue is dissolved in 400 mL methylene chloride. This solution is then added dropwise to a solution of 1,3-diaminopropane (31.7 mL, 380 mmol) and triethylamine (65.4 mL, 470 mmol) in 300 mL methylene chloride cooled in an ice/brine bath. After the addition is complete, solid has precipitated from the reaction. 200 mL additional methylene chloride is added and the reaction is stirred at room temperature under argon for 18 h. The reaction is then diluted with 600 mL methylene chloride and washed twice

with saturated sodium bicarbonate solution, once with brine, and once with 1N potassium hydroxide. The organic layer is dried over sodium sulfate, and the solvent is removed in vacuo. This solid is recrystallized from hot ethanol and washed with heptane to afford 3''-(aminopropyl)-4'-(trifluoromethyl)-2-biphenylcarboxamide.

To a stirred solution of [1-bromobutyl]-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (29.5 g, 69.2 mmol) (described in US 5,712,279) in 100 mL dimethylformamide under argon is added anhydrous potassium carbonate (9.55 g, 69.2 mmol) followed by 3''-(aminopropyl)-4'-(trifluoromethyl)-2-biphenylcarboxamide (22.3 g, 69.2 mmol). The reaction mixture is then heated to 50 C and stirred under argon for 24 h. After cooling, the reaction is filterred to remove potassium carbonate, and the filter cake is rinsed with ethyl acetate. The filtrate is partitioned between 20% heptane in ethyl acetate and water. The organic layer is washed five times with water and once with brine. The organic layer is then dried over sodium sulfate and the solvent is removed in vacuo. This solid is recrystallized from 300 mL 25% ethyl acetate in heptane to provide the desired compound to be used in the following coupling step.

A solution of 20 mmols of the above compound in DMF with 10 mmols of 1,10-dibromodecane and 20 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between ethyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

EXAMPLE 10



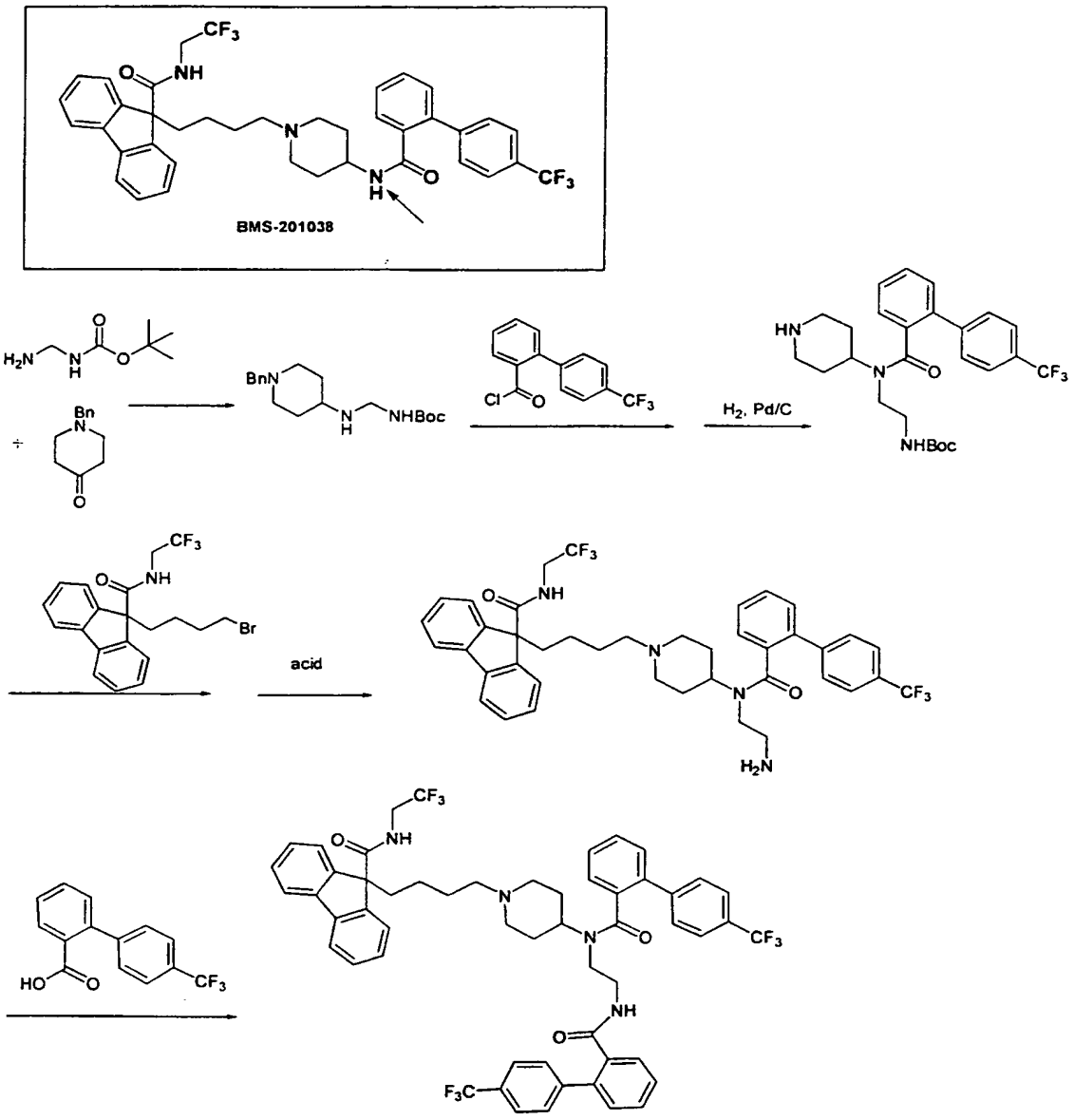
[1-Bromobutyl]-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (29.5 g, 69.2 mmol), methylamine hydrochloride (5.06 g, 75.0 mmol), and anhydrous potassium carbonate (23.0 g, 166 mmol) are placed in a glass pressure vessel. 100 mL Dimethylformamide is added, and the vessel is then sealed and heated at 50 C for 48 h, cooled and concentrated to dryness, and taken up in 500 mL methylene chloride. The solution is washed with three 80 mL portions of saturated sodium bicarbonate solution and then two 80 mL portions of brine, followed by drying over magnesium sulfate and evaporation of solvent. The crude is fractionated by flash chromatography on 600 g silica gel, loading the mixture in methylene chloride and then eluting with a step gradient of 2% to 3% methanol in methylene chloride (4 L total solvent volume). Fractions containing pure compound are combined and evaporated to yield the desired product.

A solution of 20 mmols of the above compound in DMF with 20 mmols of 1,6-dibromohexane and 20 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between ethyl acetate and

water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

A solution of 10 mmols of the above compound in DMF with 10 mmols of the compound described in US 5,712,279 and 10 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between ethyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

EXAMPLE 11



Potassium hydroxide pellets (16 g, 0.25 mole) are added to a stirred solution of *tert*-butyl *N*-(2-aminoethyl)carbamate (160 g, 1.00 mole) in 1 L of methanol. Once the pellets are completely dissolved, 1-benzyl-4-piperidone (185 mL, 1.00 mole) is added in one portion and the resulting suspension is stirred under reflux for 1 h. The reaction is then cooled in an ice bath and treated dropwise with sodium cyanoborohydride (22.0 g, 0.35 mole) in 250 mL methanol. This mixture is allowed to warm to room temperature and is then refluxed for 1 h. After cooling to room temperature, the mixture is treated with potassium hydroxide pellets (60 g, 1.5 mole) and stirred

until they are completely dissolved. The reaction mixture is suction filtered and then concentrated to 250 mL on a rotary evaporator. This is then diluted with 500 mL half-saturated brine and extracted with two 500 mL portions of diethyl ether. The combined organic layers are in turn extracted with two 500 mL portions of 2 N sodium hydrogen sulfate and then discarded. The combined aqueous extracts are adjusted to pH 10 by the addition of 6 M sodium hydroxide and then back-extracted with two 500 mL portions of ethyl acetate. The combined ethyl acetate extracts are extracted with 500 mL brine, dried over sodium sulfate, and dried to afford the crude 1-benzyl-4-[(2-aminoethylcarbamato)amino]-piperidine.

The acid chloride derivative of available 4-(trifluoromethyl)-biphenyl-2'-carboxylic acid is generated as described in US 5,712,279. To a slurry of available 4-(trifluoromethyl)-biphenyl-2'-carboxylic acid (50.0 g, 190 mmol) in 500 mL methylene chloride is added oxalyl chloride (28.7 mL, 330 mmol) followed by five drops of dimethylformamide. The reaction bubbles vigorously and is stirred at room temperature under argon for 2 h. At this time all solid has dissolved and gas evolution has ceased. The solvent is removed in vacuo, and the residue is dissolved in 400 mL methylene chloride. This solution is then added dropwise to a solution of the crude 1-benzyl-4-[(2-aminoethylcarbamato)amino]-piperidine (63.3 g, 190 mmol) prepared in the previous step and triethylamine (65.4 mL, 470 mmol) in 300 mL methylene chloride cooled in an ice/brine bath. After the addition is complete, a lot of solid has precipitated from the reaction. 200 mL additional methylene chloride is added and the reaction is stirred at room temperature under argon for 18 h. The reaction is then diluted with 600 mL methylene chloride and washed twice with saturated sodium bicarbonate solution, once with brine, and once with 1N potassium hydroxide. The organic layer is dried over sodium sulfate, and the solvent is removed in vacuo. This solid is recrystallized from hot ethanol and washed with heptane to afford 1-benzyl-4-[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl](2-aminoethylcarbamato)amino]-piperidine.

A solution of 1-benzyl-4-[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl](2-aminoethylcarbamato)amino]-piperidine (52.5 g, 100 mmol) in a mixture of 200 mL ethanol plus 10 mL glacial acetic acid is treated with 10% palladium on activated carbon (2.6 g) and then subjected to hydrogenation on a Parr apparatus (initial pressure 40 psi) for 19 h. The reaction is then filtered through celite and the filtrate is concentrated to dryness. The residue is dissolved in 500 mL chloroform and washed once with 100 mL 1 N potassium hydroxide and three times

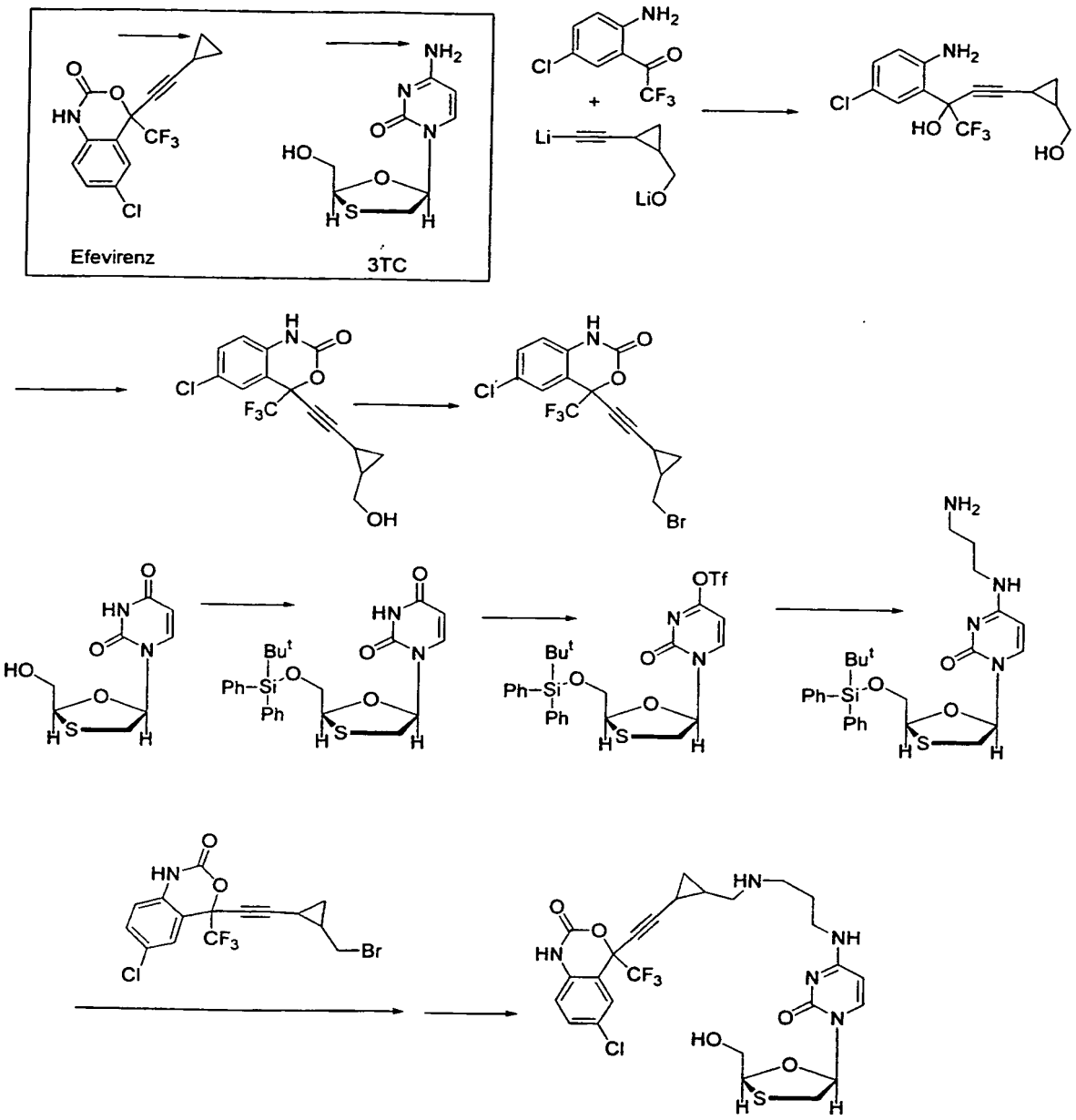
with 100 mL brine. The aqueous layers are combined and back-extracted with three 80 mL portions of chloroform. The combined organic extracts are then dried over sodium sulfate and evaporated to afford 4-[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl](2-aminoethylcarbamato)amino]-1-piperidine.

To a stirred solution of [1-bromobutyl]-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (29.5 g, 69.2 mmol) in 100 mL dimethylformamide under argon is added anhydrous potassium carbonate (9.55 g, 69.2 mmol) followed by 4-[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl](2-aminoethylcarbamato)amino]-1-piperidine prepared in the previous step (30.1 g, 69.2 mmol). The reaction mixture is then heated to 50 C and stirred under argon for 24 h. After cooling, the reaction is filtered to remove potassium carbonate, and the filter cake is rinsed with ethyl acetate. The filtrate is partitioned between 20% heptane in ethyl acetate and water. The organic layer is washed five times with water and once with brine. The organic layer is then dried over sodium sulfate and the solvent is removed in vacuo. This solid is recrystallized from 300 mL 25% ethyl acetate in heptane to provide 9-[4-[4-[[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl](2-aminoethylcarbamato)amino]-1-piperidinyl]butyl]-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide, the Boc-protected form of the desired compound.

To a solution of 9-[4-[4-[[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl](2-aminoethylcarbamato)amino]-1-piperidinyl]butyl]-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide (30.0 g, 38.5 mmol) in 100 mL dioxane is added 75 mL 4 N HCl in dioxane (300 mmol). The reaction is stirred at room temperature for 4 h, then concentrated in vacuo to afford 9-[4-[4-[[[4'-(trifluoromethyl)[1,1'-biphenyl]-2-yl]carbonyl]amino]-1-piperidinyl]butyl]-N-(2,2,2-trifluoroethyl)-9H-fluorene-9-carboxamide) as the dihydrochloride.

The above product (4.0 mmol) is dissolved in 10 mL anhydrous dimethylformamide and treated with hydroxybenzotriazole (5.0 mmol), diisopropylethyl amine (4.0 mmol), PyBOP (4.0 mmol), and 4-(trifluoromethyl)-biphenyl-2'-carboxylic acid (US 5,712,279) (4.0 mmol). The coupling reaction is stirred overnight at room temperature. Volatiles are removed under vacuum and the crude is fractionated by reverse-phase HPLC to afford the desired product after lyopholization of the appropriate fractions.

EXAMPLE 12



2-Ethynylcyclopropylmethanol (prepared as described in Tetrahedron Letters, 1992, 33, 4905) (50 mmol) is dissolved in dry THF (25 mL) at 0°. A solution of n-BuLi in hexane (100 mmol) is added. After 10 minutes, a solution of 4-chloro-2-trifluoroacetylaniline (prepared as described in WO 8904535) (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The

extract is dried and evaporated, and the residue is chromatographed to afford the intermediate carbinol.

This compound (50 mmol) is dissolved in dry THF (25 mL) and carbonyldiimidazole (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 6-chloro-1,4-dihydro-4-[2-(hydroxymethyl)cycloprop-1-ylethynyl]-4-trifluoromethyl-2H-3,1-benzoxazin-2-one.

The above compound (50 mmol) is dissolved in CH_2Cl_2 (50 mL) and CBr_4 (50 mmol) and PPh_3 (50 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The organic phase is dried and evaporated, and the residue is chromatographed to afford 4-[2-(bromomethyl)cycloprop-1-ylethynyl]-6-chloro-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one.

3-Thia-2,3-dideoxyuridine, prepared as described in US Patent 5,700,937 (5 mmol) is dissolved in DMF (100 mL) and imidazole (10 mmol) and tert-butyldiphenylsilyl chloride (6 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxyuridine.

The above compound (1 mmol) is dissolved in CH_2Cl_2 (10 mL) and pyridine (2 mL). The solution is cooled to 0° and trifluoromethanesulfonic anhydride (1 mmol) is added The mixture is left for 1 hour, and then the solvents are removed under vacuum to afford the triflate ester.

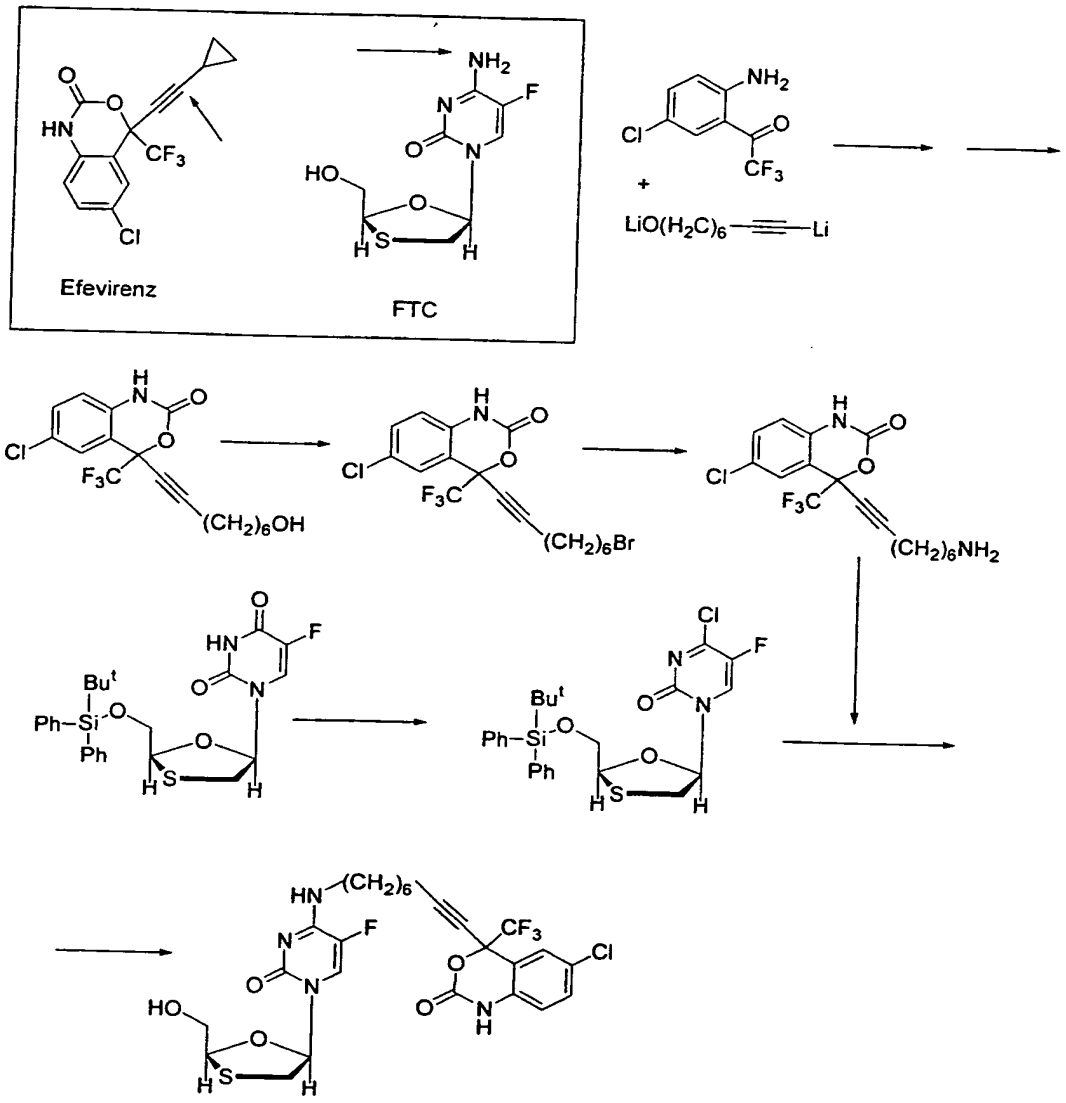
The above compound is dissolved in dry THF (5 mL) and the solution is added, with vigorous stirring, to 1,3-diaminopropane (2 mL). After 24 hours, the mixture is added to water and extracted with CH_2Cl_2; the extract is washed with water, then dried and evaporated. The residue is chromatographed to afford the desired compound.

The bromide and amine synthesized above (1 mmol each) are dissolved in DMF (15 mL) containing K_2CO_3 (250 mg) and KI (50 mg). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the silylated dimer.

The above product (1 mmol) is dissolved in THF (10 mL) and a solution of Bu_4NF (2 mmol)

in THF (2 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 and the extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

EXAMPLE 13



Oct-7-yn-1-ol (50 mmol) is dissolved in dry THF (25 mL) at 0°. A solution of n-BuLi in hexane (100 mmol) is added. After 10 minutes, a solution of 4-chloro-2-trifluoroacetylaniline,

prepared as described in WO 8904535) (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

This compound (50 mmol) is dissolved in dry THF (25 mL) and carbonyldiimidazole (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford desired compound.

The above compound (50 mmol) is dissolved in CH_2Cl_2 (50 mL) and CBr_4 (50 mmol) and PPh_3 (50 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The organic phase is dried and evaporated, and the residue is chromatographed to 4-[2-(8-bromooct-1-ynyl]-6-chloro-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one.

The above compound (100 mmol) is dissolved in THF (20 mL) and the solution is added to concentrated NH_4OH (50 mL) with vigorous stirring. The progress of the reaction is monitored by tlc. When it is complete, the mixture is extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

[5'-Tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxy-5-fluorouridine is prepared as follows: Using a procedure similar to that described in US Patent 5,700,937, 2-(tert-butyldiphenylsilyloxy)methyl-5-acetoxy-1,3-oxathiolane, prepared as described in the aforementioned patent, (2 mmol) is dissolved in CH_2Cl_2 (50 mL) and a premixed solution of silylated 5-fluorouracil, prepared as described in US Patent 5,700,937 (2 mmol) and 1M $SnCl_4$ in CH_2Cl_2 (4 ml, 4 mmol) is added over 30 minutes. After 6 hours, pyridine (3 mL) is added, and the solvents are removed under vacuum. The residue is dissolved in ethanol, and is then evaporated to low volume. The residue is chromatographed to afford 5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxy-5-fluorouridine.]

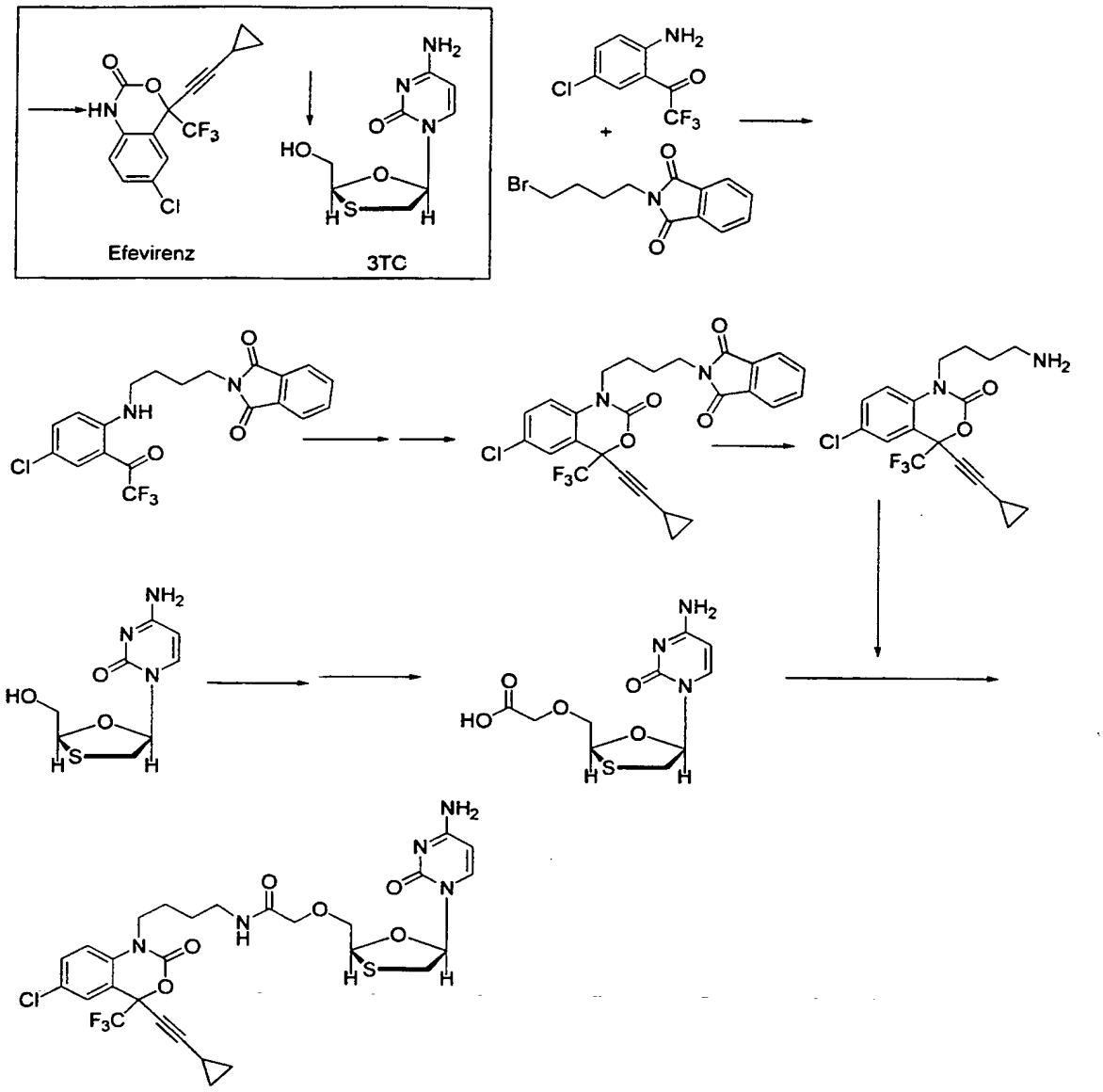
The above compound (5 mmol) is dissolved in CH_2Cl_2 (50 mL) and thionyl chloride (50 mmol) and DMF (0.1 mL) are added. The solution is heated under reflux for 2 hour, then is cooled. The volatile components are removed under vacuum to afford the desired compound.

4-[2-(6-Aminooct-1-ynyl]- 6-chloro-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one (5 mmol) is dissolved in EtOH (50 mL) and the chloroimine above (5 mmol) is added. The

mixture is heated at reflux while the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and then added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

The above product (1 mmol) is dissolved in THF (10 mL) and a solution of Bu_4NF (2 mmol) in THF (2 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 and the extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

EXAMPLE 14



4-Chloro-2-trifluoroacetylaniline (100 mmol) and 4-bromobutylphthalimide (50 mmol) are heated at reflux in EtOH (100 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and added to water. The aqueous solution is extracted with EtOAc, and the residue is dried and evaporated. The residue is chromatographed to afford N-(4-phthalimidobutyl)-4-chloro-2-trifluoroacetylaniline.

Cyclopropylacetylene (50 mmol) is dissolved in dry THF (25 mL) at 0°. A solution of n-BuLi in hexane (100 mmol) is added. After 10 minutes, a solution of 4-chloro-2-

trifluoroacetylaniline (prepared as described in WO 8904535) (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

This compound (50 mmol) is dissolved in dry THF (25 mL) and carbonyldiimidazole (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-1-(4-phthalimidobutyl)-4-trifluoromethyl -2H-3,1-benzoxazin-2-one.

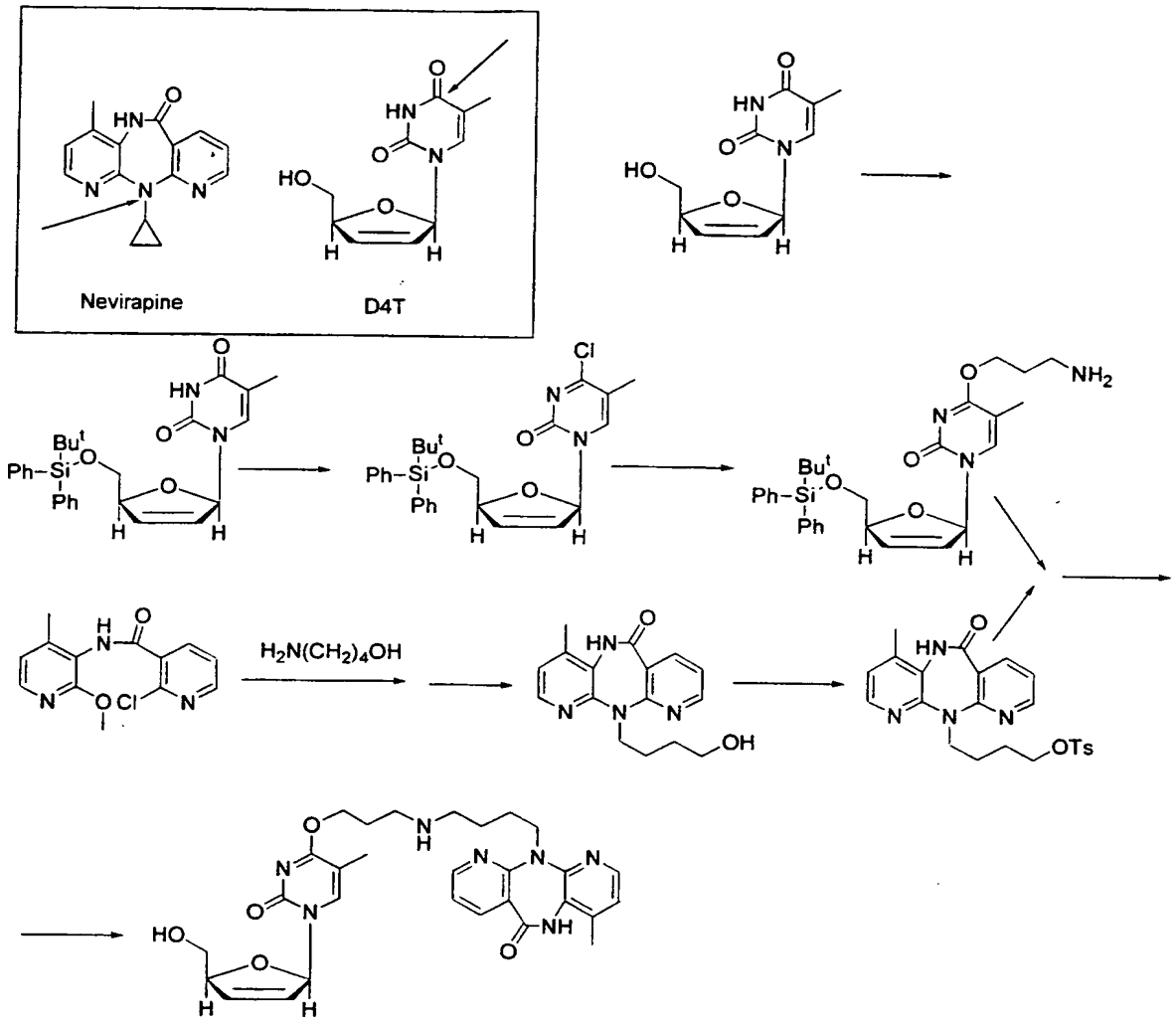
The above compound (50 mmol) is dissolved in EtOH (100 mL) and 85% hydrazine hydrate (200 mmol) is added. The mixture is heated at reflux while the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and it is then poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

3'-Thia-2',3'-dideoxycytidine (5 mmol) is dissolved in DMF (30 mL) and methyl bromoacetate (5 mmol), K_2CO_3 (0.5g) and KI (50 mg) are added. The mixture is heated to 50°, and the progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-(carbomethoxymethyl)-3'-thia-2',3'-dideoxycytidine.

The above compound (1 mmol) is dissolved in THF (5 mL) and a solution of LiOH (1.5 mmol) in water (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into dilute HCl. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated, and chromatographed.

1-(4-Aminobutyl)-6-chloro-1,4-dihydro-4-(cyclopropylethynyl)-4-trifluoromethyl-2H-3,1-benzoxazin-2-one (2 mmol) and the 3TC-derived carboxylic acid above (2 mmol) are dissolved in DMF (20 mL) containing dicyclohexylcarbodiimide (3 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric compound.

EXAMPLE 15



D4T (5 mmol) is dissolved in DMF (100 mL) and imidazole (10 mmol) and tert-butyldiphenylsilyl chloride (6 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-tert-butyldiphenylsilyloxy-2',3'-didehydro-3'-deoxythymidine.

The above compound (5 mmol) is dissolved in CH_2Cl_2 (50 mL) and thionyl chloride (50 mmol) and DMF (0.1 mL) are added. The solution is heated under reflux for 2 hour, then is cooled. The volatile components are removed under vacuum to afford the into the chloroimine compound.

Sodium hydride (2 mmol) is added to a solution of 3-aminopropanol (2 mmol) in DMF (15

mL). When hydrogen evolution has stopped, a solution of the chloroimine above (1 mmol) in DMF (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

2-Chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, prepared as described in J. Org. Chem., 1995, 60, 1875, (5 mmol) and 4-aminobutanol (10 mmol) are heated at 110° in a sealed tube for 16 hours. The excess amine is removed under vacuum and the residue is chromatographed to afford the desired compound.

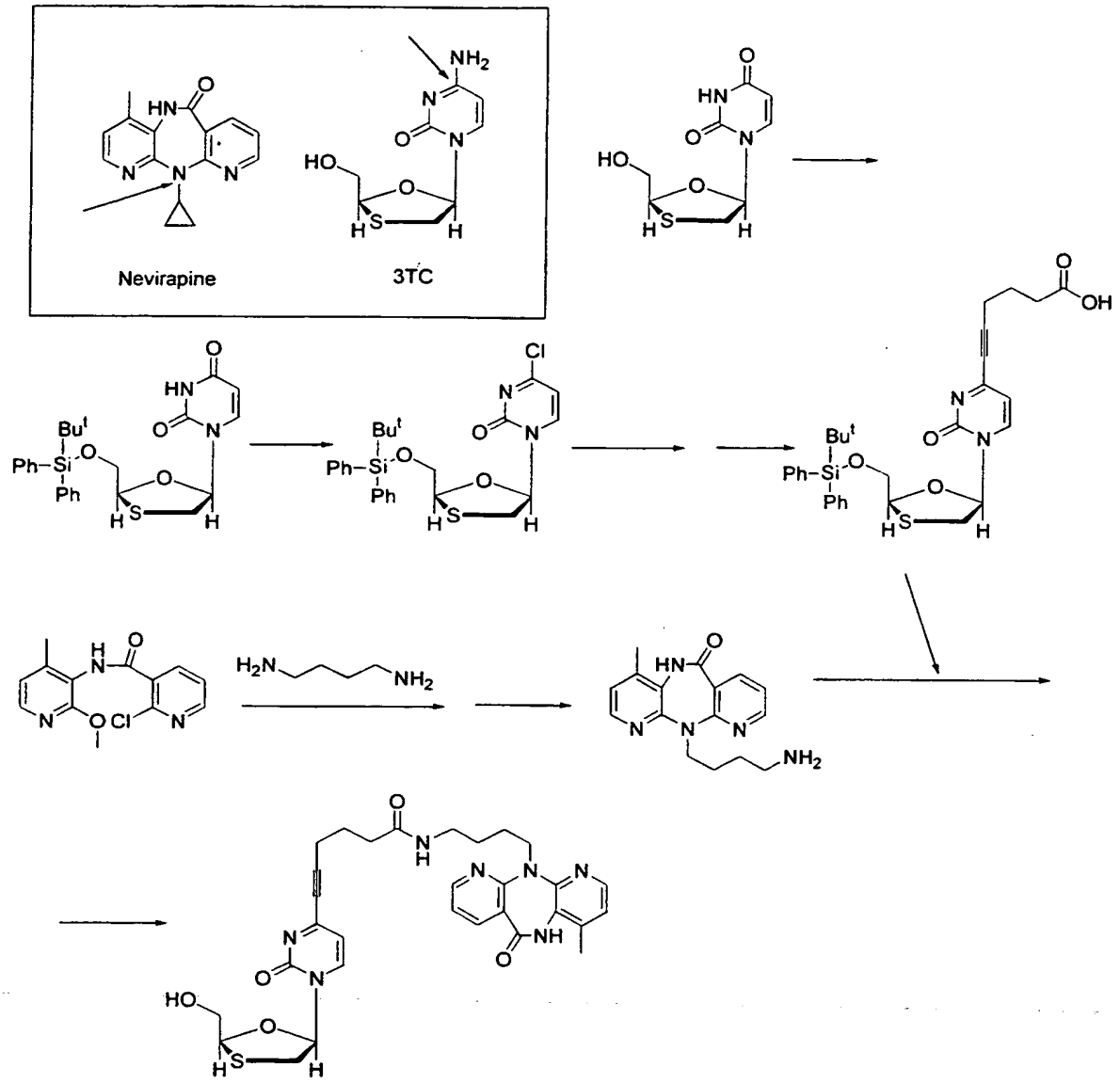
The above compound (1 mmol) is dissolved in pyridine (3 mL) and a 1M solution of sodium hexamethyldisilazide (3 mmol) is added. The mixture is heated to 60°; the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and it is then poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

The compound so obtained (5 mmol) is dissolved in pyridine (50 mL) and p-toluenesulfonyl chloride (6 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5,11-dihydro-4-methyl-11-[4-(p-toluenesulfonyloxy)butyl]-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.

This tosylate (1 mmol) and the amine prepared above (1 mmol)are dissolved in DMF (15 mL) containing K_2CO_3 (250 mg) and KI (50 mg). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the silylated dimer.

The above product (1 mmol) is dissolved in THF (10 mL) and a solution of Bu_4NF (2 mmol) in THF (2 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric compound.

EXAMPLE 16



3-Thia-2,3-dideoxyuridine, prepared as described in US Patent 5,700,937 (5 mmol) is dissolved in DMF (100 mL) and imidazole (10 mmol) and tert-butyldiphenylsilyl chloride (6 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxyuridine.

The above compound (5 mmol) is dissolved in CH_2Cl_2 (50 mL) and thionyl chloride (50

mmol) and DMF (0.1 mL) are added. The solution is heated under reflux for 2 hour, then is cooled. The volatile components are removed under vacuum.

Using a procedure similar to that described in J. Het. Chem., 1994, 31, 989, $(Ph_3P)_2PdCl_2$ (0.3g), and CuI (75mg) are added to dry THF (100 mL) under an inert atmosphere. Et_3N (3 mL) and methyl hex-5-ynoate (20 mmol) are then added. After 10 minutes, a solution of the above product (10 mmol) in THF (25 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is diluted with CH_2Cl_2 (100 mL). The solution is washed with dilute HCl, then dried and evaporated. The residue is chromatographed to afford the coupled product.

The above compound (1 mmol) is dissolved in THF (5 mL) and a solution of LiOH (1.5 mmol) in water (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into dilute HCl. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford the desired compound.

2-Chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide prepared as described in J. Org. Chem., 1995, 60, 1875, (5 mmol) and 1,4-diaminobutane (10 mmol) are heated at 110° in a sealed tube for 16 hours. The excess amine is removed under vacuum and the residue is chromatographed to afford 2-[2-(hydroxymethyl)cyclopropylamino]-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide.

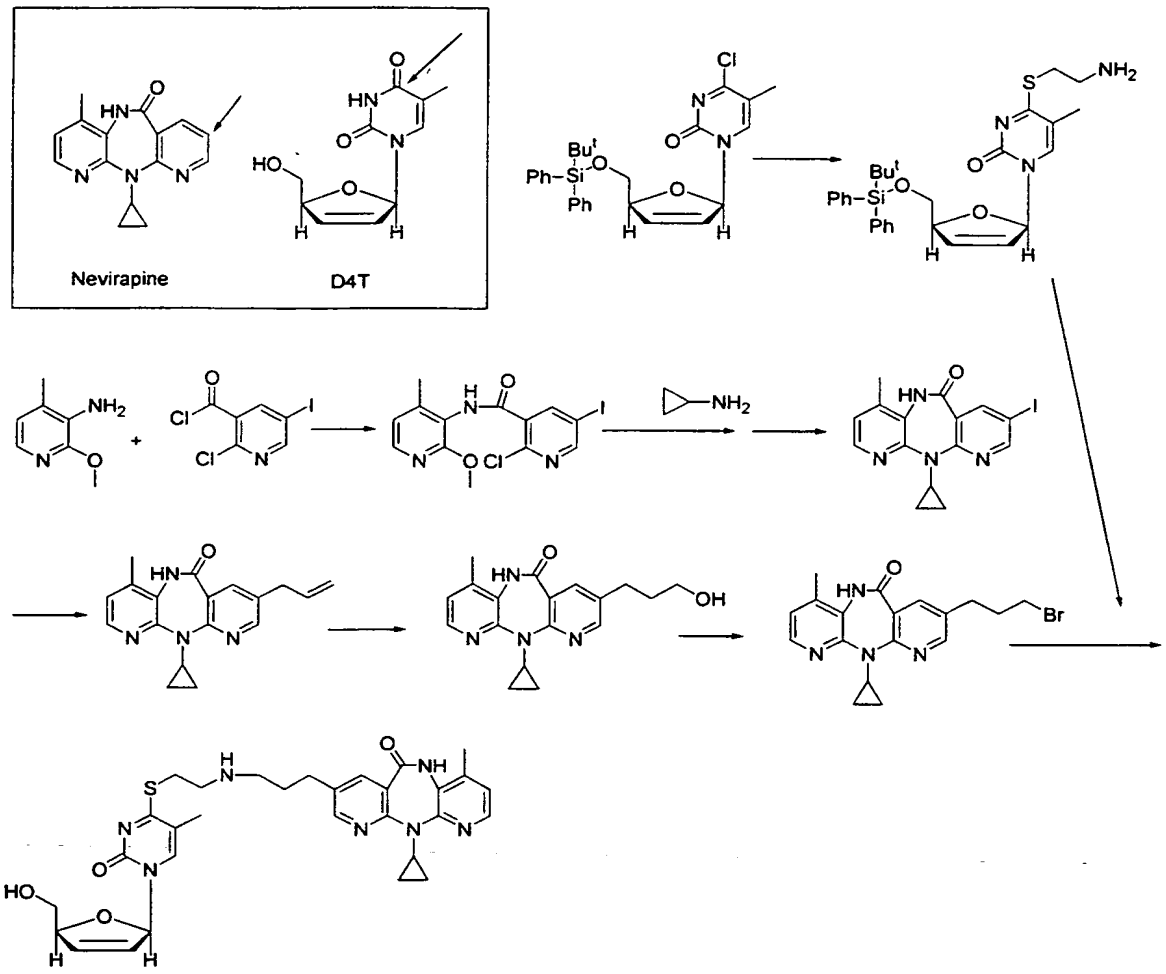
The above compound (1 mmol) is dissolved in pyridine (3 mL) and a 1M solution of sodium hexamethyldisilazide (3 mmol) is added. The mixture is heated to 60°; the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and it is then poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

The above product (2 mmol) and the carboxylic acid prepared above (2 mmol) are dissolved in DMF (20 mL) containing dicyclohexylcarbodiimide (3 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2, and the extract is dried and evaporated. The residue is chromatographed to afford the dimeric compound.

The above product (1 mmol) is dissolved in THF (10 mL) and a solution of Bu_4NF (2 mmol) in THF (2 mL) is added. The progress of the reaction is monitored by tlc. When it is complete,

the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2, and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric compound.

EXAMPLE 17



2-Mercaptoethylamine (25 mmol) is dissolved in EtOH (50 mL) and the chloroimine from Example 15 (5 mmol) is added. The mixture is heated at reflux while the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and then added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the thioether compound.

3-Amino-2-methoxy-4-methylpyridine (5 mmol) and 2-chloro-5-iodopyridine-3-carbonyl chloride, prepared as described in J. Med. Chem., 1997, 40, 2674, or J. Chem. Eng. Data, 1976,

21, 246, are coupled under conventional amide coupling conditions. The product (10 mmol) and aminocyclopropane (10 mmol) are heated at 110° in a sealed tube for 16 hours. The excess amine is removed under vacuum and the residue is chromatographed to afford 2-[2-(hydroxymethyl)cyclopropylamino]-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide.

The above compound (1 mmol) is dissolved in pyridine (3 mL) and a 1M solution of sodium hexamethyldisilazide (3 mmol) is added. The mixture is heated to 60°; the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and it is then poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is dried and evaporated. The residue is chromatographed to afford the compound 11-cyclopropyl-5,11-dihydro-8-iodo-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.

Using a procedure similar to that described in J. Med. Chem., 1998, 41, 2960, the above compound (2 mmol) is dissolved in DMF (15 mL) and allyltributylstannane (2 mmol) and $(PPh_3)_4Pd$ (0.1 mmol) are added. The mixture is heated to 60°. The progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and 0.1M aqueous KF (10 mL) is added. After 1 hour, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 8-allyl-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.

The above compound (1 mmol) is dissolved in THF (10 mL) and diborane in THF (1 mmol) is added. After 6 hours, 30% H_2O_2 (1 mmol), and 0.1 N NaOH (10 mL) are added. The mixture is left for 24 hours, then is acidified by the addition of dilute HCl. The pH is adjusted to 10 by addition of dilute NaOH, and the mixture is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5,11-dihydro-8-(3-hydroxypropyl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.

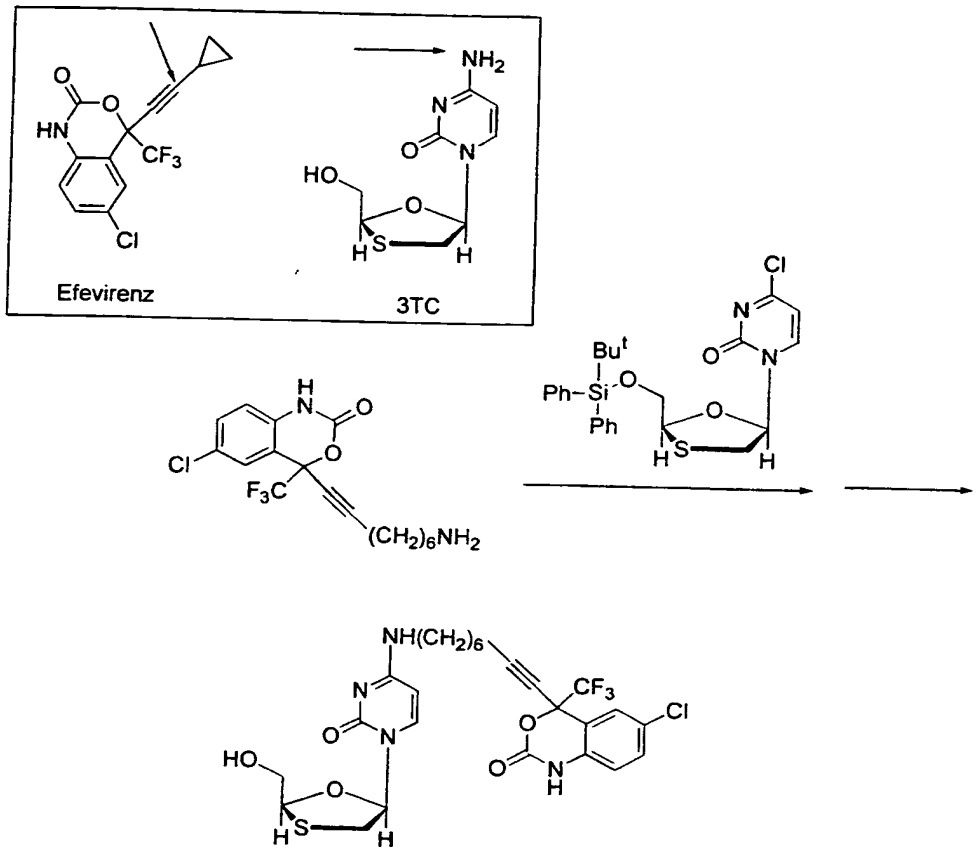
The compound (50 mmol) is dissolved in CH_2Cl_2 (50 mL) and CBr_4 (50 mmol) and PPh_3 (50 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The organic phase is dried and evaporated, and the residue is chromatographed to afford the desired compound.

The amine prepared above (1 mmol) and 8-(3-bromopropyl)-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (1 mmol) are dissolved in DMF (15 mL) containing K_2CO_3 (250 mg) and KI (50 mg). The progress of the reaction is monitored by tlc.

When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the silylated dimer.

The above product (1 mmol) is dissolved in THF (10 mL) and a solution of Bu_4NF (2 mmol) in THF (2 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the compound desired dimeric compound.

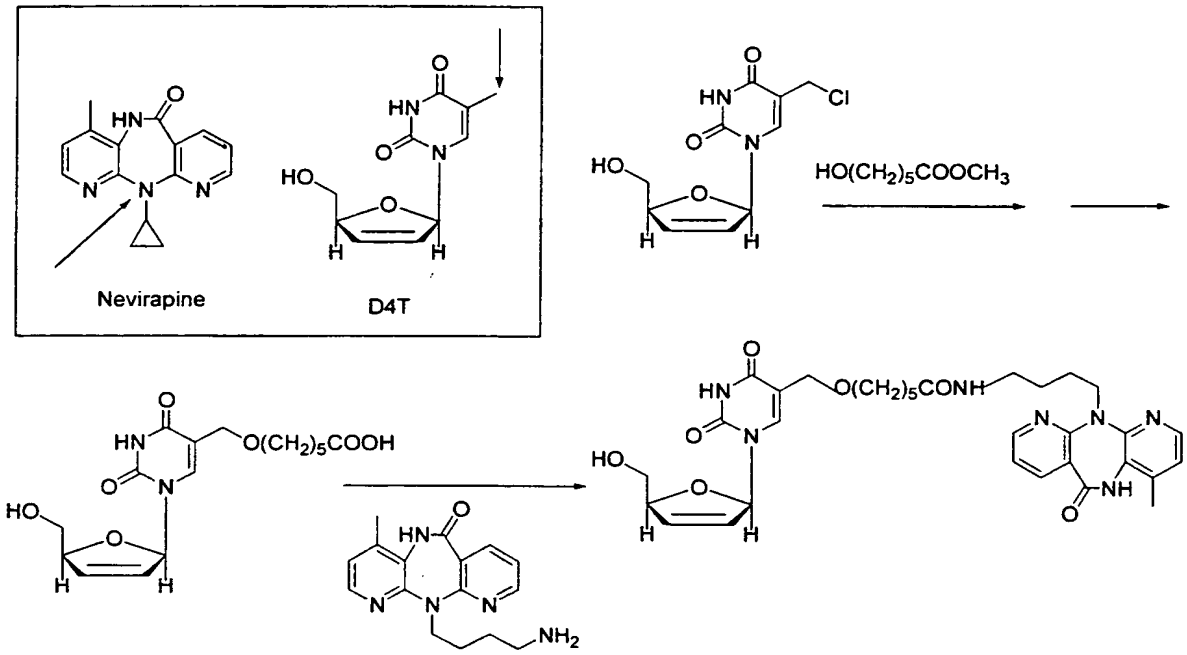
EXAMPLE 18



6-Chloro-4-[2-(6-aminooct-1-ynyl]-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one (25 mmol), prepared as in Example 13, is dissolved in EtOH (50 mL) and the chloroimine from Example 16 (5 mmol) is added. The mixture is heated at reflux while the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and then added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the dimeric product.

The product (1 mmol) is dissolved in THF (10 mL) and a solution of Bu_4NF (2 mmol) in THF (2 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric compound.

EXAMPLE 19

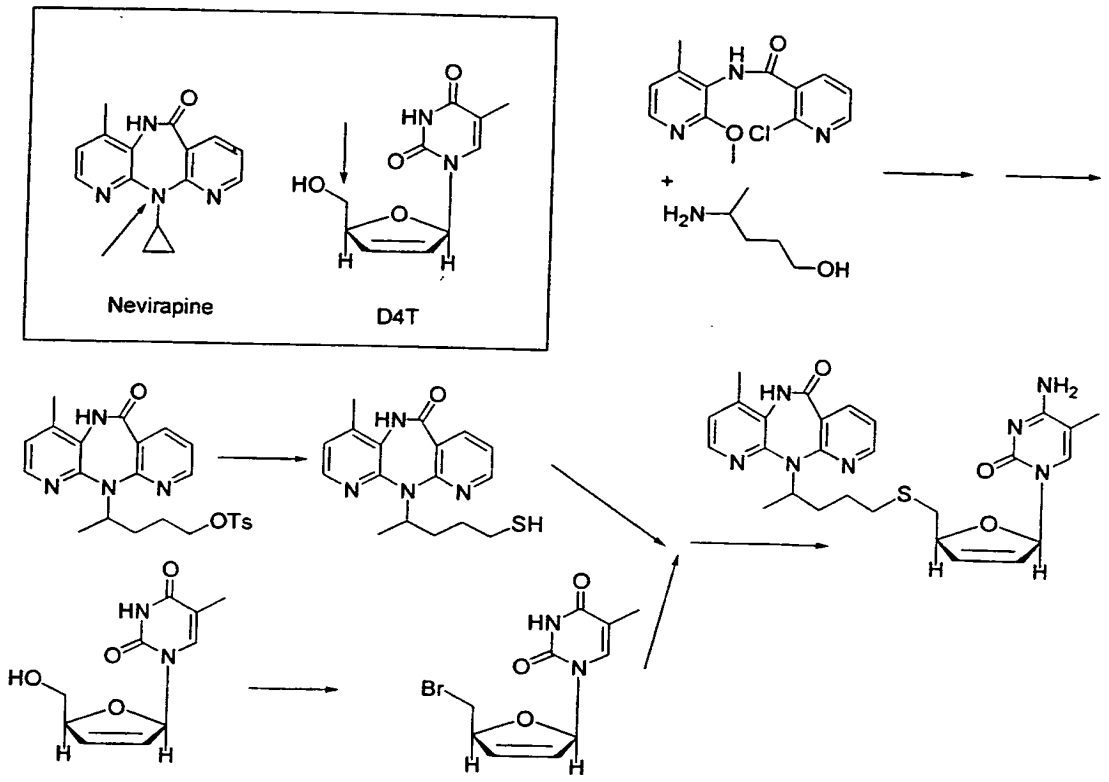


Sodium hydride (1 mmol) is added to a solution of methyl 6-hydroxyhexanoate (1 mmol) in DMF (20 mL) When hydrogen evolution has ceased, a solution of the chloromethyl compound, prepared as described in Antiviral Agents and Chemotherapy, 9, 205, (1 mmol) in DMF (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the methyl ester of the acid.

The above compound (1 mmol) is dissolved in THF (5 mL) and a solution of LiOH (1.5 mmol) in water (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into dilute HCl. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford the desired carboxylic acid.

11-(4-aminobutyl)-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (2 mmol) (prepared in Example 16) and the D4T carboxylic acid (2 mmol) are dissolved in DMF (20 mL) containing dicyclohexylcarbodiimide (3 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric compound.

EXAMPLE 20



2-Chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, prepared as described in J. Org. Chem., 1995, 60, 1875, (5 mmol) and 4-aminopentanol (10 mmol) are heated at 110° in a sealed tube for 16 hours. The excess amine is removed under vacuum and the residue is chromatographed to afford the desired compound.

The above compound (1 mmol) is dissolved in pyridine (3 mL) and a 1M solution of sodium hexamethyldisilazide (3 mmol) is added. The mixture is heated to 60°; the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and it is then poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is dried and evaporated. The residue is chromatographed to afford the desired compound.

The compound so obtained (5 mmol) is dissolved in pyridine (50 mL) and p-toluenesulfonyl chloride (6 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5,11-dihydro-4-methyl-11-[5-(p-toluenesulfonyloxy)pent-2-yl]-6H-dipyrido[3,2-b:2',3'-

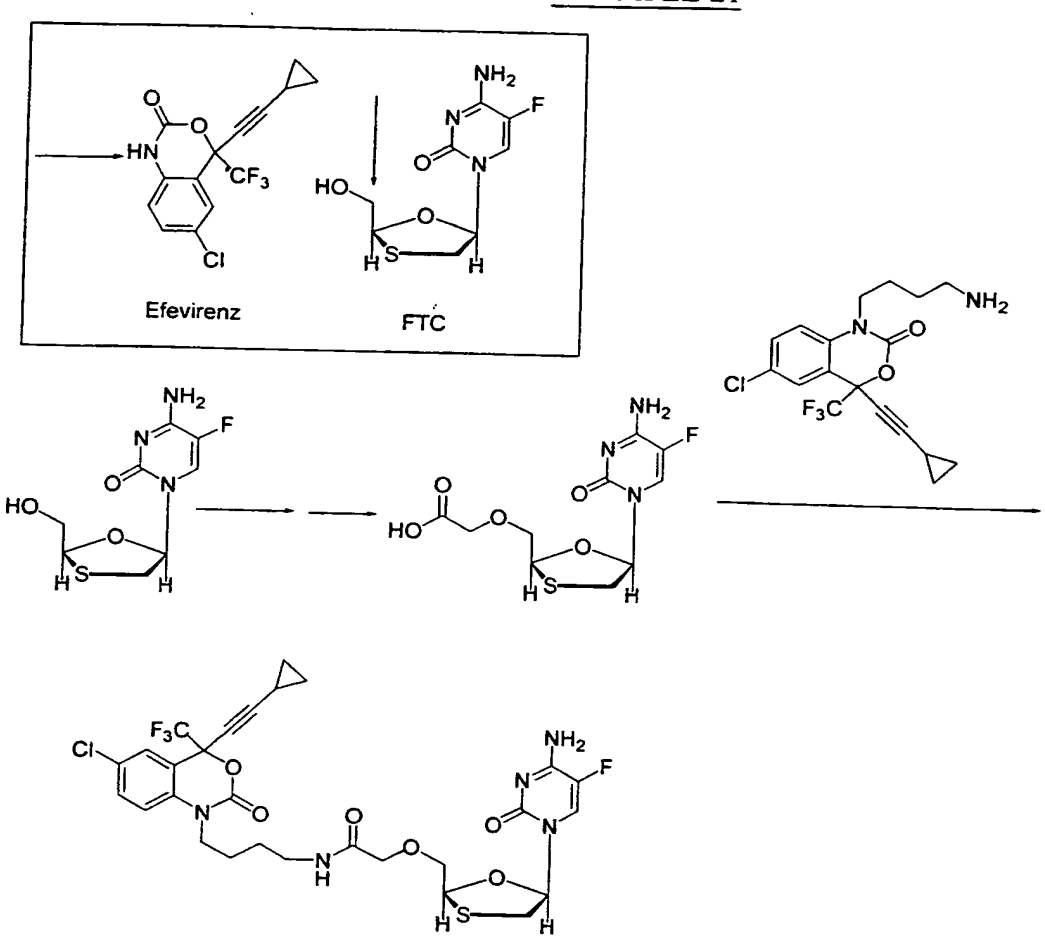
e][1,4]diazepin-6-one.

The above compound (50 mmol) is dissolved in EtOH (50 mL) and thiourea (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water, and extracted with EtOAc. The extract is dried and evaporated, and the residue redissolved in EtOH (50 mL). Tetramethylene pentamine (200 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 5,11-dihydro-11-(5-mercaptopent-2-yl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.

D4T (50 mmol) is dissolved in CH_2Cl_2 (50 mL) and CBr_4 (50 mmol) and PPh_3 (50 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The organic phase is dried and evaporated, and the residue is chromatographed to afford 5'-bromo-2',3'-didehydro-3'-deoxythymidine.

5,11-dihydro-11-(5-mercaptopent-2-yl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (1 mmol) is dissolved in DMSO (10 mL) and 5'-bromo-2',3'-didehydro-3'-deoxythymidine (1 mmol) and diisopropylethylamine (5 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric product.

EXAMPLE 21

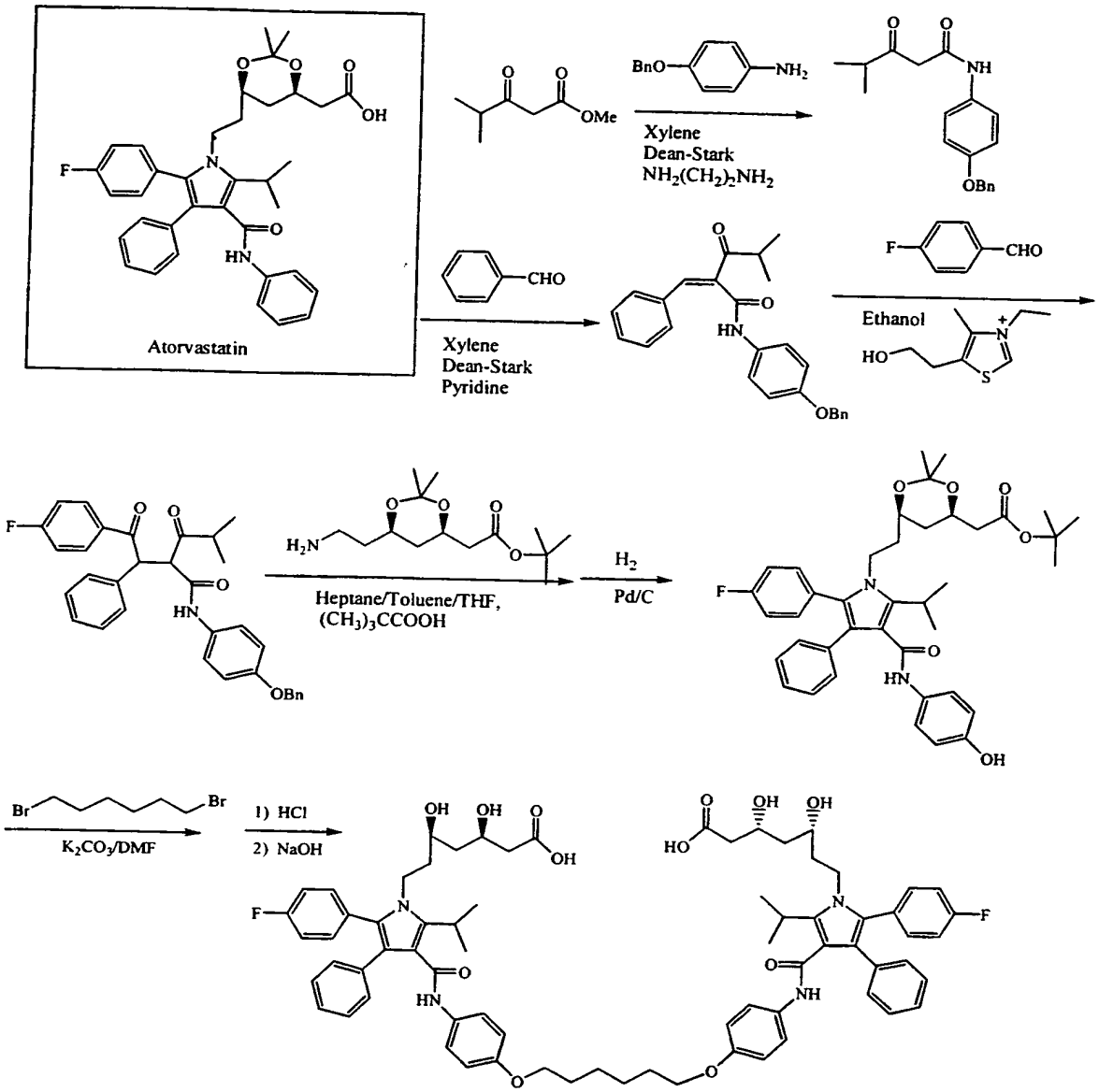


3'-Thia-2',3'-dideoxy-5-fluorocytidine (FTC) (5 mmol) is dissolved in DMF (30 mL) and methyl bromoacetate (5 mmol), K_2CO_3 (0.5g) and KI (50 mg) are added. The mixture is heated to 50°, and the progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford the desired compound.

The above compound (1 mmol) is dissolved in THF (5 mL) and a solution of LiOH (1.5 mmol) in water (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into dilute HCl. The aqueous solution is extracted with CH_2Cl_2. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-carboxymethyl-3'-thia-2',3'-dideoxy-5-fluorocytidine.

1-(4-Aminobutyl)-6-chloro-1,4-dihydro-4-(cyclopropylethynyl)-4-trifluoromethyl-2H-3,1-benzoxazin-2-one (2 mmol) (prepared in Example 14) and 5'-carboxymethyl-3'-thia-2',3'-dideoxy-5-fluorocytidine (2 mmol) are dissolved in DMF (20 mL) containing dicyclohexylcarbodiimide (3 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the desired dimeric compound.

EXAMPLE 22

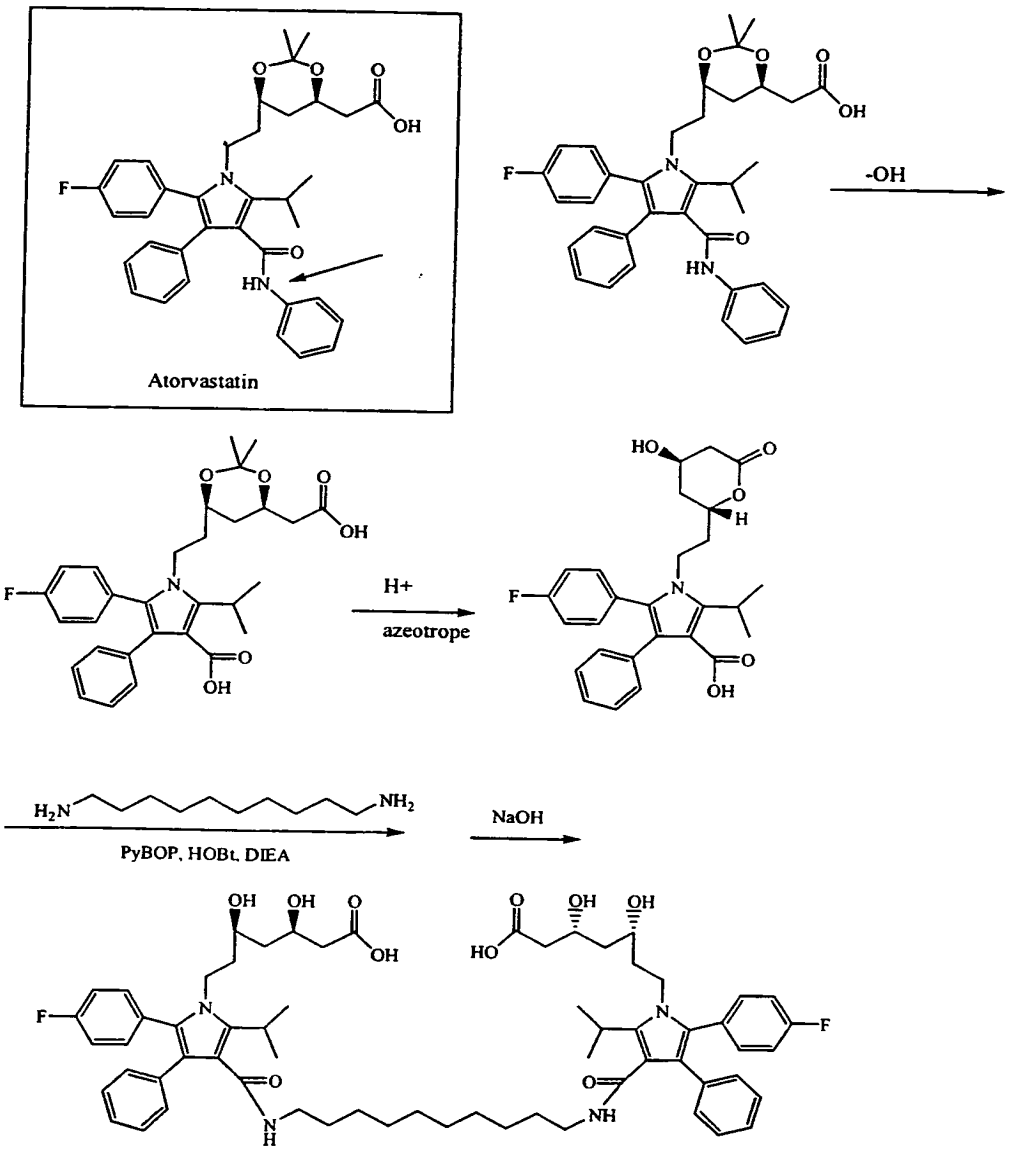


4-Benzoxyaniline is reacted with methylisobutyryl acetate in the presence of 1,2 ethylene diamine. The reaction is conducted for about 16 hours in refluxing xylenes, using a Dean-Stark trap. The resulting amide is reacted *in situ* with benzaldehyde to provide a keto amide intermediate. The reaction is conducted in refluxing xylenes, again using a Dean-Stark Trap to remove the water produced in the condensation reaction. The desired condensation product is isolated using conventional techniques, such as recrystallization or chromatography. The product is then reacted with 4-fluorobenzaldehyde in ethanol in the presence of triethylamine and

a catalytic amount of 3-ethyl-5-(2-hydroxyethyl)-4-methylthaizolium bromide. The reaction is conducted at about 90 C for about 12 hours. The diketone intermediate is then condensed with (4R-cis)-1,1-dimethylethyl-6-(2-aminoethyl)-2,2-dimethyl-1,3-dioxane-4-acetate (prepared as described in US Patent No. 5,216,174 and EPO 330172A2). The reaction is conducted by heating in the presence of pivalic acid in an inert diluent comprising heptane/toluene/THF (4:1:1 by volume) for about 48 hours. These reactions are describd in greater detail in US 5,385,929 and Tet. Lett. 1992, *33*, 2279. The resulting pyrrole is then removed using conventional technique, such as hydrogenolysis in the presence of a catalyst such as palladium on carbon. The product is isolated and purified by conventional technique.

A solution of the alcohol prepared above (671 mg, 1.0mmol) and 1,6-dibromohexane (122mg, 0.5mmol) in DMF (2mL) is treated with finely powdered potassium carbonate (276mg, 2.0mmol) and the mixture is stirred at 60°C for 18 hours. Water is added, and the product extracted into chloroform. The solvent is removed in vacuo to provide the desired product. The product of the preceding reaction is dissolved in methanol (5mL) and 1N HCl (5mL) and stirred at room temperature for 18 hours. The reaction mixture is concentrated and treated with THF/MeOH/1N NaOH (10mL). After 4 hours at room temperature, the reaction is concentrated, water (10 mL) is added, and the solution is washed with ether. The aqueous layer is acidified with 1N HCl, and extracted with ethyl acetate. The organic layer is dried, filtered and concentrated. The residue is purified by reverse-phase HPLC using a gradient of 0.1% TFA/acetonitrile in 0.1% aqueous TFA to afford the desired product.

EXAMPLE 23



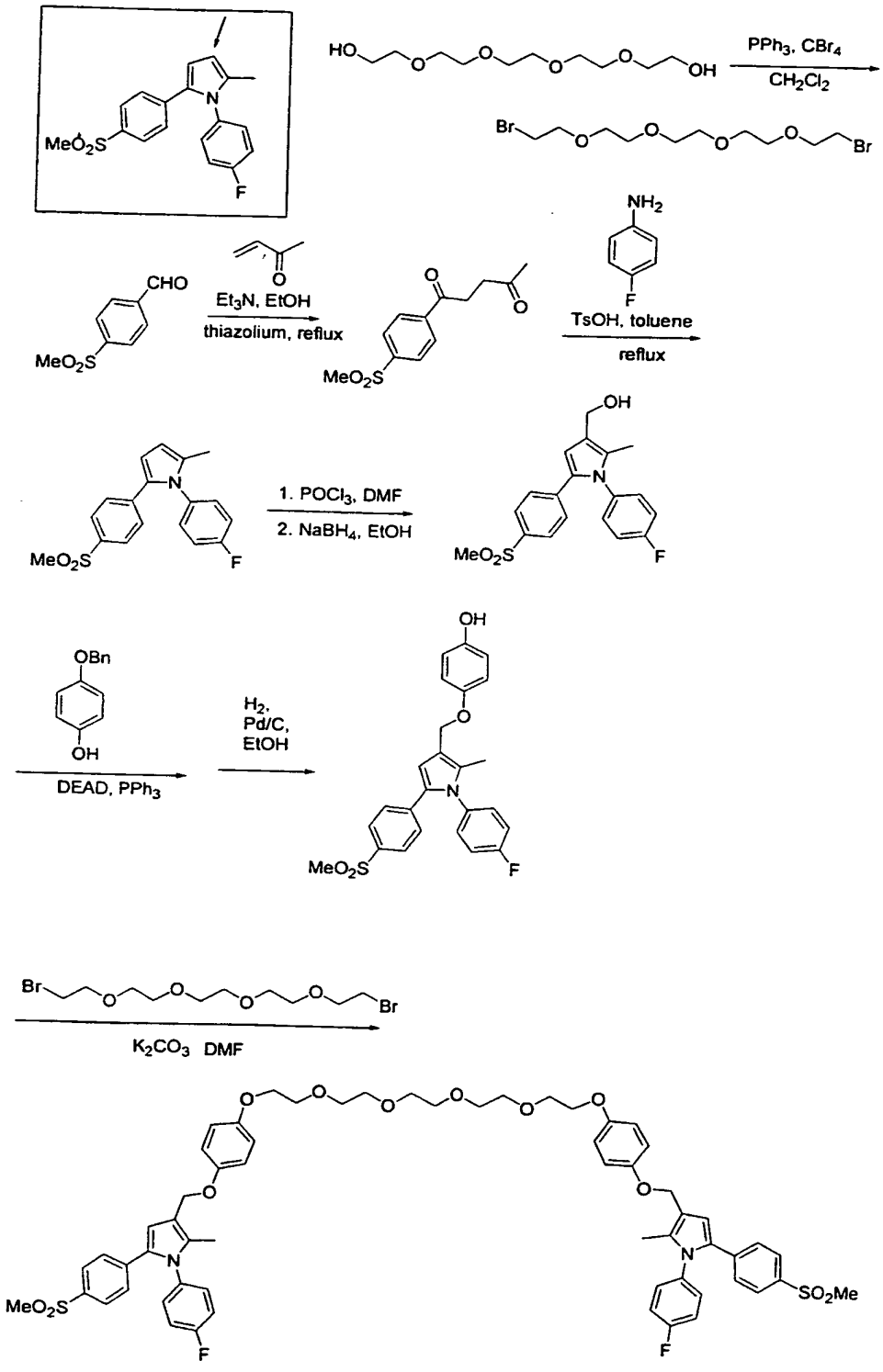
The N-phenyl amide moiety of atorvastatin (prepared, for example, according to US 5,397,792 and 5,446,054) is hydrolyzed by reacting atorvastatin with an excess of an alkali or alkaline earth metal hydroxide, such as sodium hydroxide, in an inert diluent, such as ethanol. After completion of the hydrolysis reaction, the product is acidified to form the lactone intermediate.

To a solution of this intermediate (465 mg, 1.0mmol) and 1,10-diaminodecane (86mg, 0.5mmol) in DMF (100mL) is added N,N-diisopropylethylamine (258mg, 2.0mmol), N-

hydroxybenzotriazole (135mg, 1.0mmol) and PyBOP (570mg, 1.1mmol). The reaction is stirred at room temperature for 2 hours, at which time the solvent is removed in vacuo. The residue is dissolved in ethyl acetate and washed succesively with 1N HCl, saturated aqueous sodium bicarbonate, and brine, then dried and concentrated to provide the desired product.

The above product is treated with THF/MeOH/1N NaOH (10mL). After 4 hours at room temperature, the reaction is concentrated, water (10 mL) is added, and the solution washed with ether. The aqueous layer is acidified with 1N HCl, and extracted with ethyl acetate. The organic layer is dried, filtered and concentrated. The residue is purified by reverse-phase HPLC using a gradient of 0.1% TFA/acetonitrile in 0.1% aqueous TFA to afford the desired product.

EXAMPLE 24



Pentaethylene glycol (2 mmol) is dissolved in dry CH_2Cl_2 (25 mL) and triphenylphosphine (4 mmol) and carbon tetrabromide (4 mmol) are added. The reaction is monitored by tlc, and when it is complete, the mixture is evaporated to dryness. The desired product is obtained by purification by HPLC.

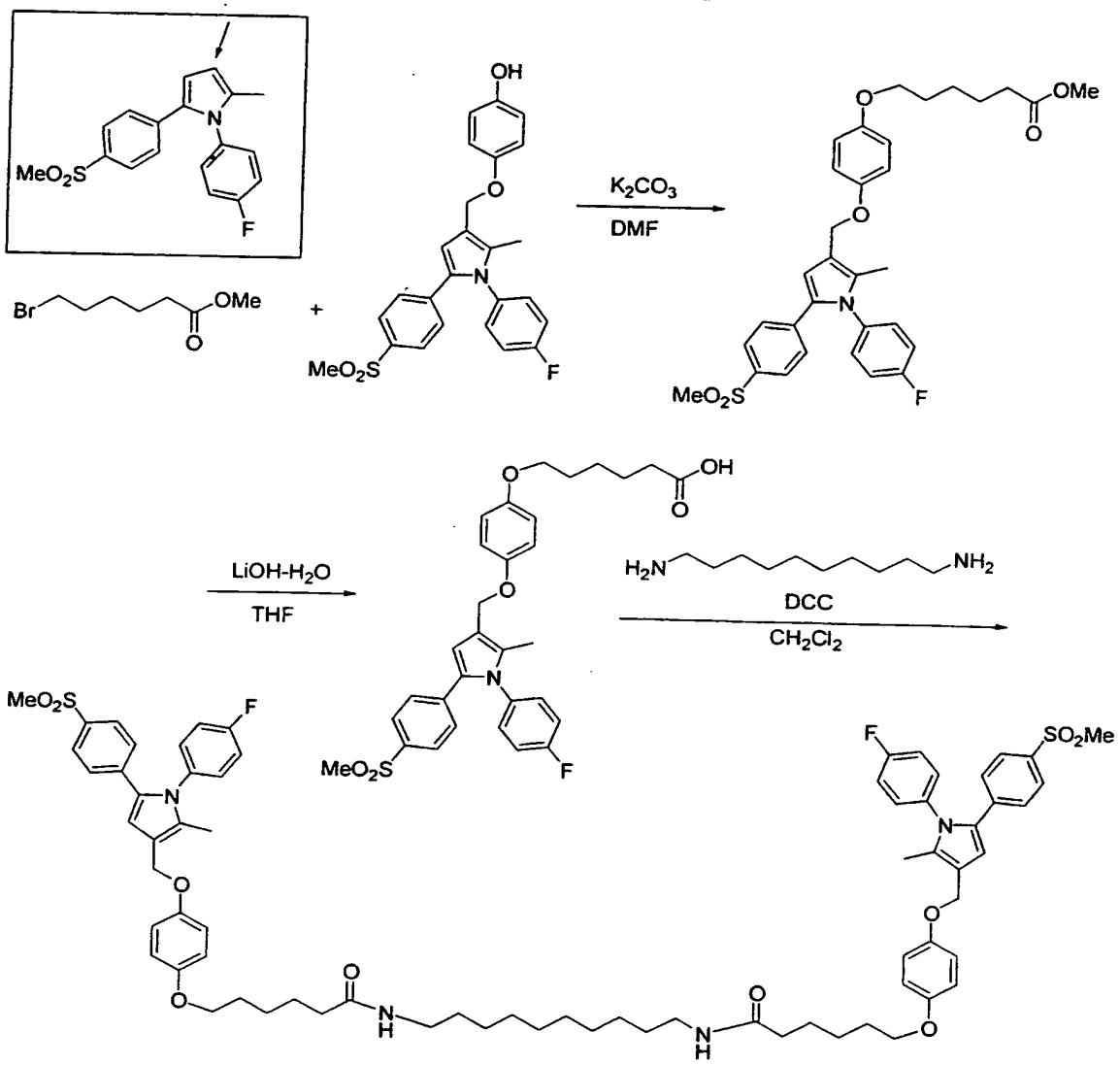
To a solution of 1-(4-fluorophenyl)-2-methyl-5-(4-methylsulfonylphenyl)pyrrole (1 mmol), (prepared in the first two steps showhn, as described in J. Med. Chem. 1997, 40, 1619), in dry DMF (10 mL) at 0 °C is added phosphorus oxychloride (1 mmol). The reaction is monitored by tlc, and when it is complete, the mixture is poured onto ice and made basic with aqueous sodium hydroxide. The product is isolated by extraction with CH_2Cl_2. The extract is dried and evaporated to afford a residue. To the residue, dissolved in EtOH (10 mL) is added sodium borohydride (100 mg). After one hour, the solution is added to water and extracted with ethyl acetate. The extract is washed with dilute HCl, dried and evaporated. The desired compound, 1-(4-fluorophenyl)-3-(1-hydroxyl)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole, is purified and isolated by HPLC.

The above product (1 mmol) is added to a solution of diethylazodicarboxylate (1 mmol) and triphenylphosphine (1 mmol) in dry THF (10 mL). 4-Benzyloxyphenol (1 mmol) is then added, and the progress of the reaction is monitored by tlc. When the reaction is complete, water is added and the product is extracted with ethyl acetate. The extract is dried and evaporated and the residue is purified by HPLC to afford the desired compound.

The above compound (1 mmol) is dissolved in EtOH (25 mL) and 10% Pd/C (25 mg) is added. The reaction mixture is stirred under an atmosphere of H_2 and monitored by tlc. When the reaction is complete, the solution is filtered and evaporated to afford a residue which, after purification by HPLC, affords 1-(4-fluorophenyl)-3-(4-hydroxyphenoxy)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole.

To a mixture of K_2CO_3 (1 g) and 1-(4-fluorophenyl)-3-(1-hydroxyl)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole (2 mmol) in dry DMF at 50 °C is added a solution of the dibromide (1 mmol), prepared as described in the reaction above, in DMF (5 mL). The reaction is monitored by tlc, and when it is complete, the mixture is added to water. The product is extracted with ethyl acetate, and the extract is dried and evaporated. The residue is purified by HPLC to afford the desired product.

EXAMPLE 25



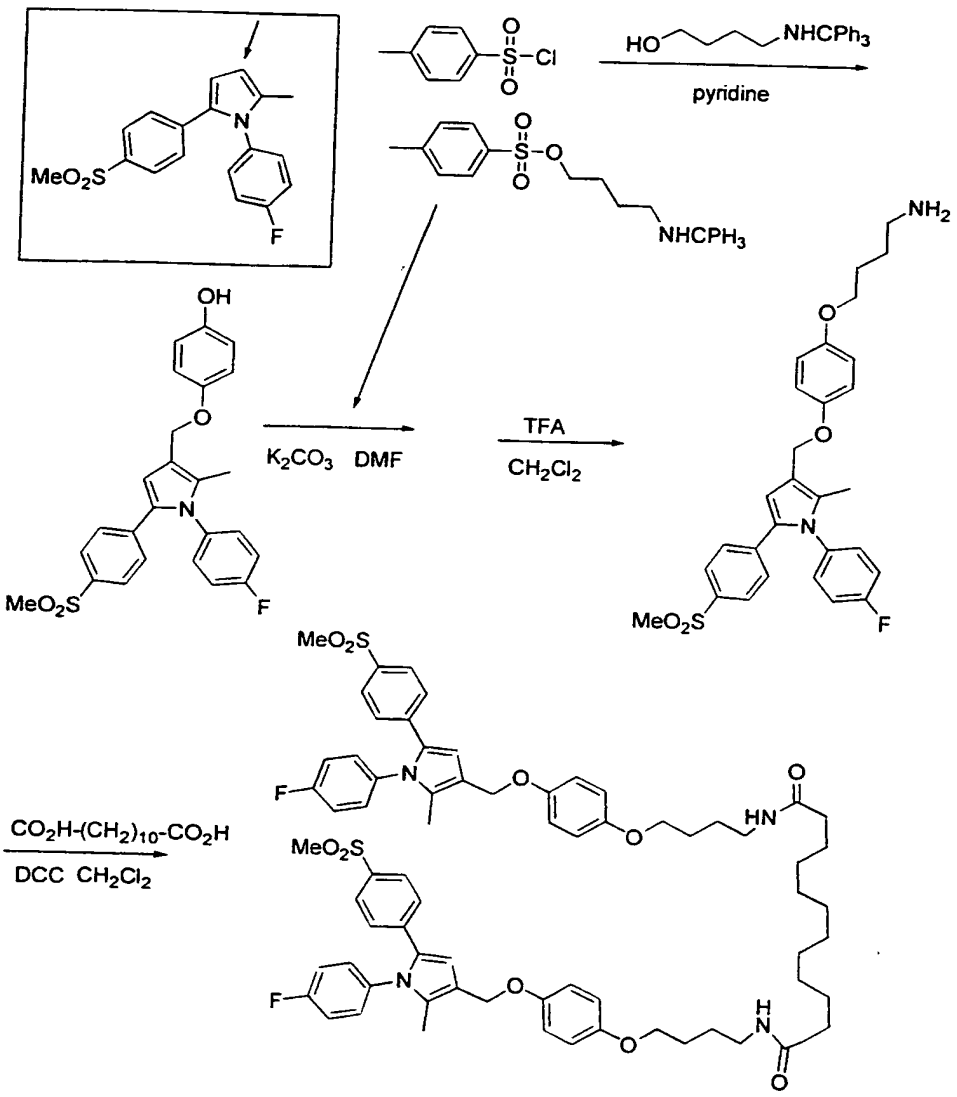
To a solution of methyl 6-bromohexanoateate (1 mmol) in DMF (20 mL) is added K_2CO_3 (1 g) and then a solution of 1-(4-fluorophenyl)-3-(4-hydroxyphenoxy)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole (1 mmol), prepared as described in Example 24, in DMF (5 mL). The mixture is stirred at room temperature and monitored by tlc. Once the reaction is complete, the mixture is added to water. The aqueous solution is extracted with ethyl acetate, the extract is dried and evaporated, and the product is purified by HPLC.

The compound from the preceding reaction (0.5 mmol) is dissolved in THF (10 mL) and a solution of LiOH-H_2O (0.55 mmol) in water (3 mL) is added. The reaction is monitored by tlc and, when it is complete, the solution is neutralized by addition o f aqueous NaH_2PO_4.

The mixture is extracted with CH_2Cl_2 and the extract is dried and evaporated. The crude product is purified by HPLC to afford the desired product.

The compound from the preceding reaction (1 mmol) is dissolved in dry CH_2Cl_2 (25 mL) and dicyclohexylcarbodiimide (1 mmol) is added, followed by 1,10-diaminodecane (0.5 mmol). The progress of the reaction is followed by tlc. When the reaction is complete, the solution is washed with dilute HCl, dilute aqueous $NaHCO_3$, then dried and evaporated. The desired compound is obtained by purification by HPLC.

EXAMPLE 26



1-Hydroxy-4-triphenylmethylaminobutane (5 mmol) is dissolved in pyridine (10 mL) and p-toluenesulfonyl chloride (5 mmol) is added. The reaction is followed by tlc. When the reaction is complete, the solution is added to water and extracted with CH_2Cl_2. The solution is washed with dilute HCl, then dried and evaporated to afford the desired compound.

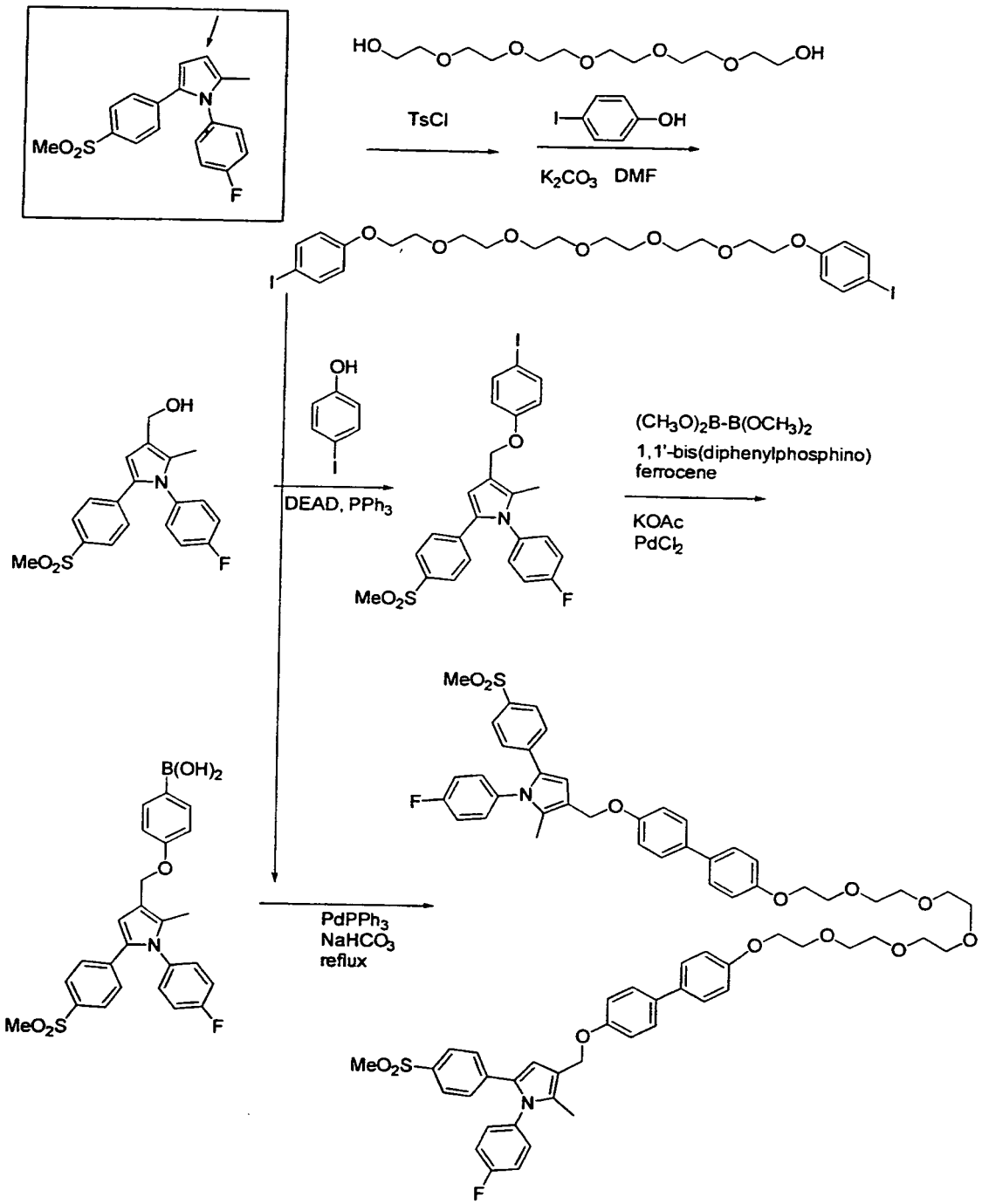
1-(4-Fluorophenyl)-3-(4-hydroxyphenoxy)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole (prepared in Example 24) (1 mmol) is dissolved in dry DMF (10 mL) containing K_2CO_3 (250 mg) and the compound prepared above (1 mmol) is added. The

reaction is followed by tlc. When the reaction is complete, the solution is poured into water and extracted with CH_2Cl_2. The extract is dried and evaporated and the product is purified by HPLC to afford the desired compound.

The triphenylmethyl-protected compound (0.5 mmol) is dissolved in CH_2Cl_2 (10 mL) at 0 °C and trifluoracetic acid (3 mL) is added. The progress of the reaction is followed by tlc. When the reaction is complete, the mixture is poured onto ice and extracted with CH_2Cl_2. The extract is washed with dilute $NaHCO_3$, then dried and evaporated. Purification by HPLC affords the desired compound.

Dicyclohexylcarbodiimide (1 mmol) is dissolved in dry CH_2Cl_2 (25 mL) and dodecanedicarboxylic acid (.25 mmol) is added, followed by the product from the preceding reaction (0.5 mmol). The reaction is followed by tlc, and when it is complete, the solution is added to water and extracted with CH_2Cl_2. The extract is washed with dilute aqueous NaHCO3, then dried and evaporated. The residue is purified by HPLC to afford the desired compound.

EXAMPLE 27



Hexaethylene glycol (5 mmol) is dissolved in pyridine (30 mL) and p-toluenesulfonyl chloride (10 mmol) is added. The progress of the reaction is followed by tlc, and when complete, the solution is added to dilute HCl and the product is extracted with ether. The extract

is dried and evaporated to afford the bis(p-toluenesulfonyl) derivative of hexaethylene glycol.

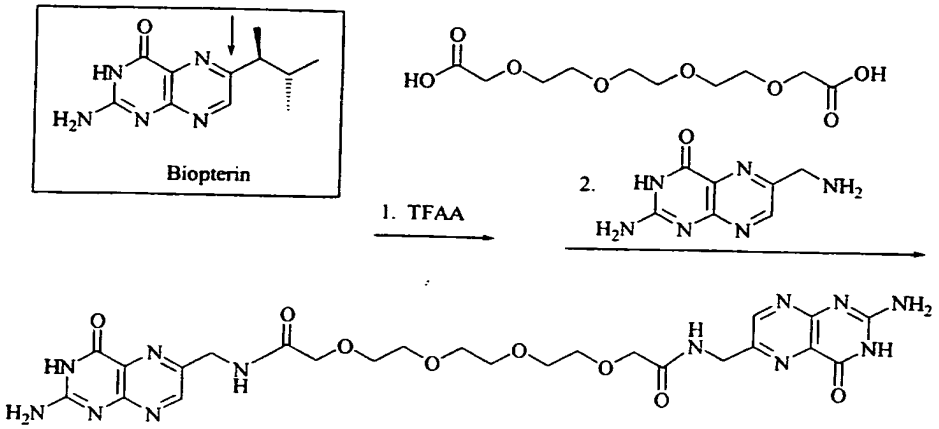
The above product (1 mmol) is dissolved in dry DMF (10 mL) and K_2CO_3 (1 g) and 4-iodophenol (2 mmol) are added. The progress of the reaction is monitored by tlc. When the reaction is complete, the solution is added to water and the product is extracted with ethyl acetate. The extract is dried and evaporated, ant the product is purified by HPLC to afford the desired bis(iodophenoxy) product.

1-(4-Fluorophenyl)-3-(1-hydroxyl)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole (1 mmol) (prepared as in Example 24) is added to a solution of diethylazodicarboxylate (1 mmol), triphenylphosphine (1 mmol) and 4-iodophenol (1 mmol) in dry THF (25 mL). The progress of the reaction is monitored by tlc. When the reaction is complete, water is added and the crude product is extracted with ethyl acetate. The extract is dried and evaporated. The residue is purified by HPLC to afford the desired compound.

The above compound (1 mmol), tetramethyl bisboronate (1 mmol), KOAc (3 mmol) and $PdCl_2$ 1,1'-bis(diphenylphosphino)ferrocene (0.03 mmol) are heated in DMSO (25 mL) at 80 °C. The progress of the reaction is monitored by tlc. When the reaction is complete, the mixture is cooled and added to water. The aqueous solution is made basic by addition of aqueous NaOH. After 3 hours, the mixture is brought to pH 1 by addition of dilute HCl, and is then extracted with CH_2Cl_2. The extract is dried and evaporated. The residue is purified by HPLC to afford 1-(4-fluorophenyl)-3-(4-boronophenoxy)methyl-2-methyl-5-(4-methylsulfonylphenyl)pyrrole.

The compound prepared in the preceding reaction (0.5 mmol), tetrakis(triphenylphosphine)Pd(0) (25 mg), the bis(iodophenoxy) compound (0.25 mmol), and $NaHCO_3$ (50 mg) are refluxed under an inert atmosphere in a mixture of toluene (10 mL), ethanol (1 mL) and water (1 mL). The progress of the reaction is followed by tlc. When the reaction is complete, the solution is filtered and the solvent is removed under vacuum. The residue is purified by HPLC to afford the desired compound.

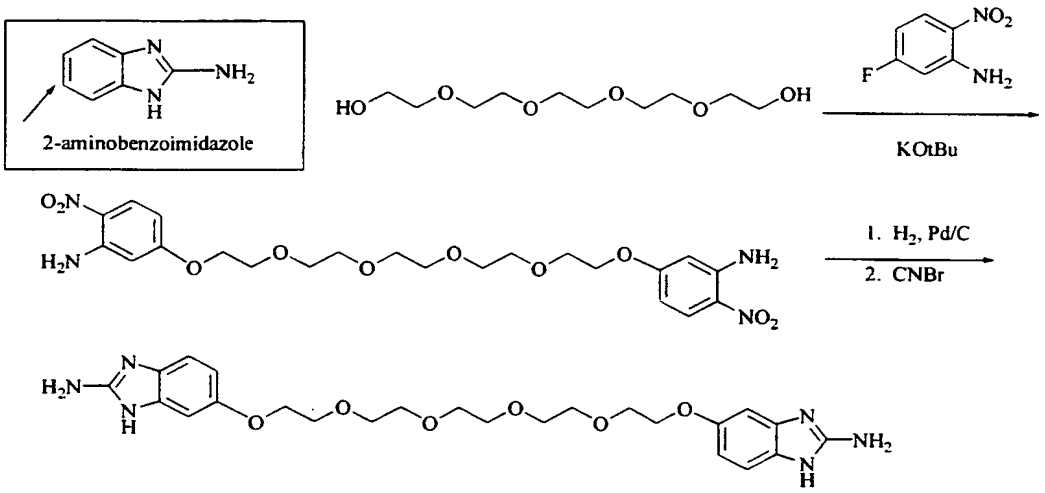
EXAMPLE 28



A solution of 20 mmols of the diacid, which is reported in JP 61,189,247; Appl. 85/28,307; CA 106: 49617c, in 100 mL of THF with 80 mmols of triethylamine is treated at room temperature with 40 mmols of trifluoroacetic anhydride. After 1 hr., a solution of 40 mmols of the amine, which is reported in Koster, S. *et al.* Eur. J. Biochem., 1995, 231, 414, is added and the reaction followed by TLC. When it is judged complete, the mixture is concentrated and the residue partitioned between water and ethyl acetate. After washing with water and sat. sodium carbonate, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

4-nitrophenyl chloroformate. After 1hr., 20 mmols of o-phenylenediamine is added and the reaction heated to reflux and followed by TLC. When judged complete, the mixture is concentrated and partitioned between sat. sodium carbonate and isopropyl acetate. After washing with water and sat. sodium carbonate, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

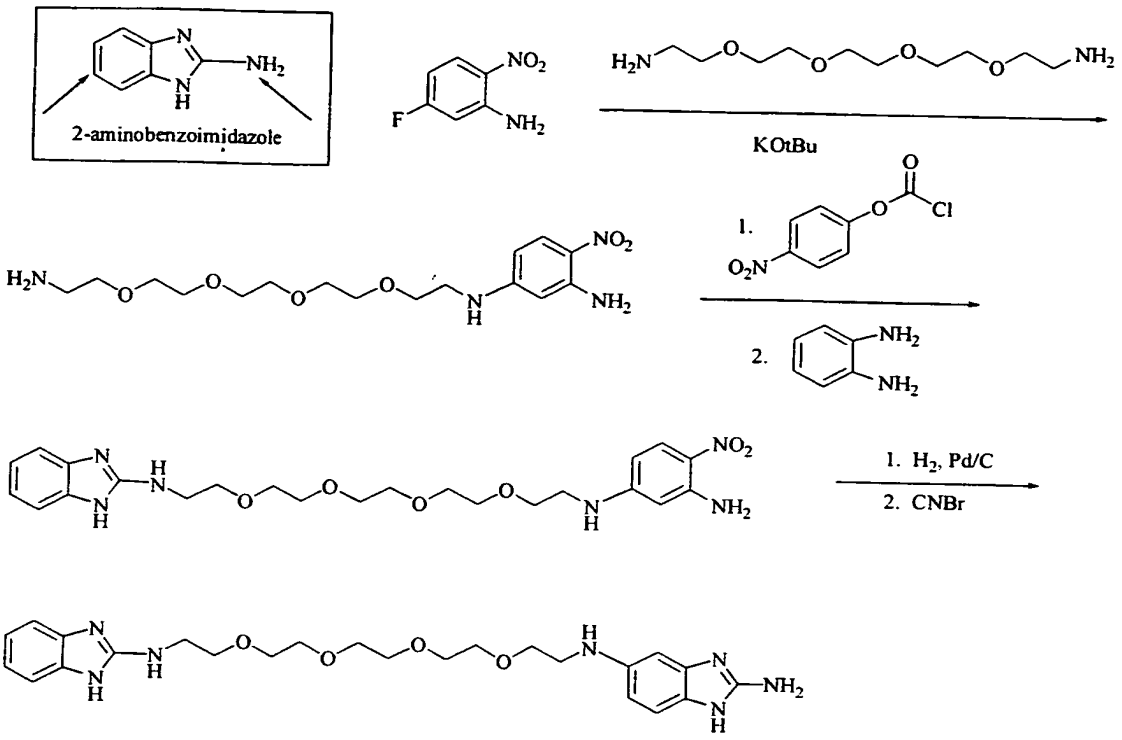
EXAMPLE 30



A mixture of 20 mmols of penta(ethylene glycol), 38 mmols of 5-fluoro-2-nitroaniline and 40 mmols potassium t-butoxide is warmed as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between water and isopropyl acetate. After washing with water, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the nitroaniline intermediate.

This material is hydrogenated at atm. pres. in the usual manner in ethanol with 10% Pd/C and followed by TLC. When judged complete, the mixture is filtered and to the filtrate is added two equivalents of cyanogen bromide and two equivalents of triethylamine and the mixture heated to reflux. When judged complete by TLC, the mixture is concentrated and the residue partitioned between ethyl acetate and water. After washing with water, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

EXAMPLE 31



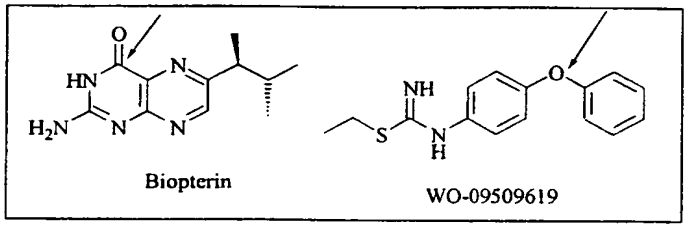
A mixture of 10 mmols of 1,14-diamino-3,6,9,12-tetraoxatetradecane (CAS 68960-97-4), 10 mmols of 5-fluoro-2-nitroaniline and 10 mmols potassium t-butoxide in 10 mL of dioxane is warmed as necessary and the reaction followed by TLC. When judged complete, the mixture is concentrated and the residue partitioned between ethyl acetate and water. After washing with water, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the mono alkylated intermediate.

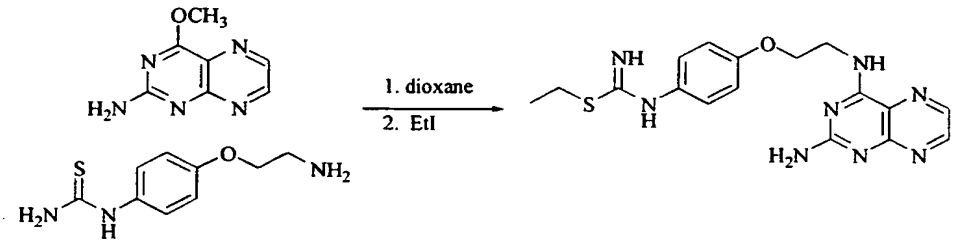
A solution of 20 mmols of the above intermediate in 25 mL of THF with 40 mmols of triethylamine is treated at room temperature with 20 mmols of 4-nitrophenyl chloroformate. After 1hr., 20 mmols of o-phenylenediamine is added and the reaction heated to reflux and followed by TLC. When judged complete, the mixture is concentrated and partitioned between sat. sodium carbonate and isopropyl acetate. After washing with water and sat. sodium carbonate, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the mono benzimidazole intermediate.

This material is hydrogenated at atm. pres. in the usual manner in ethanol with 10% Pd/C and followed by TLC. When judged complete, the mixture is filtered and to the filtrate is added one

equivalent of cyanogen bromide and one equivalent of triethylamine and the mixture heated to reflux. When judged complete by TLC, the mixture is concentrated and the residue partitioned between ethyl acetate and water. After washing with water, the organic layer is dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

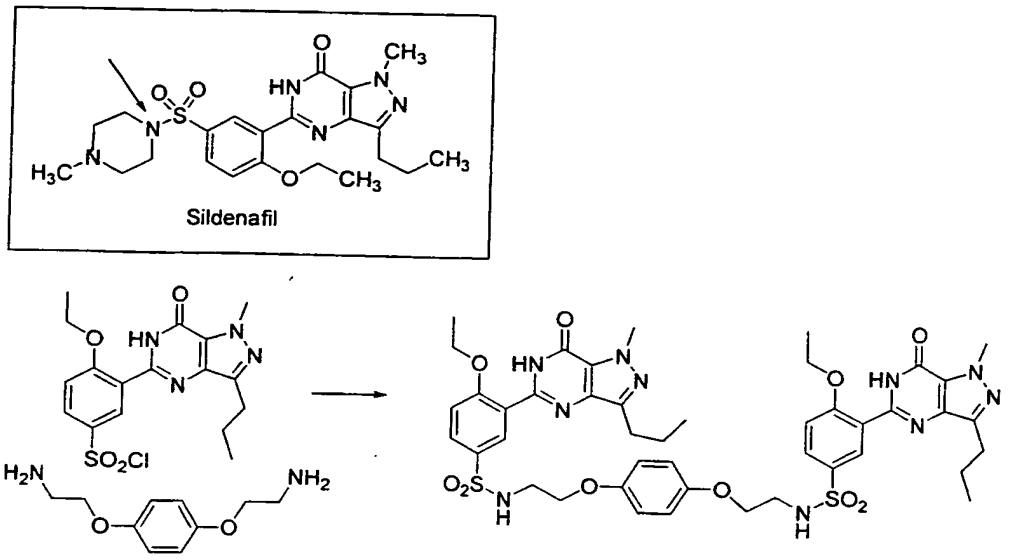
EXAMPLE 32





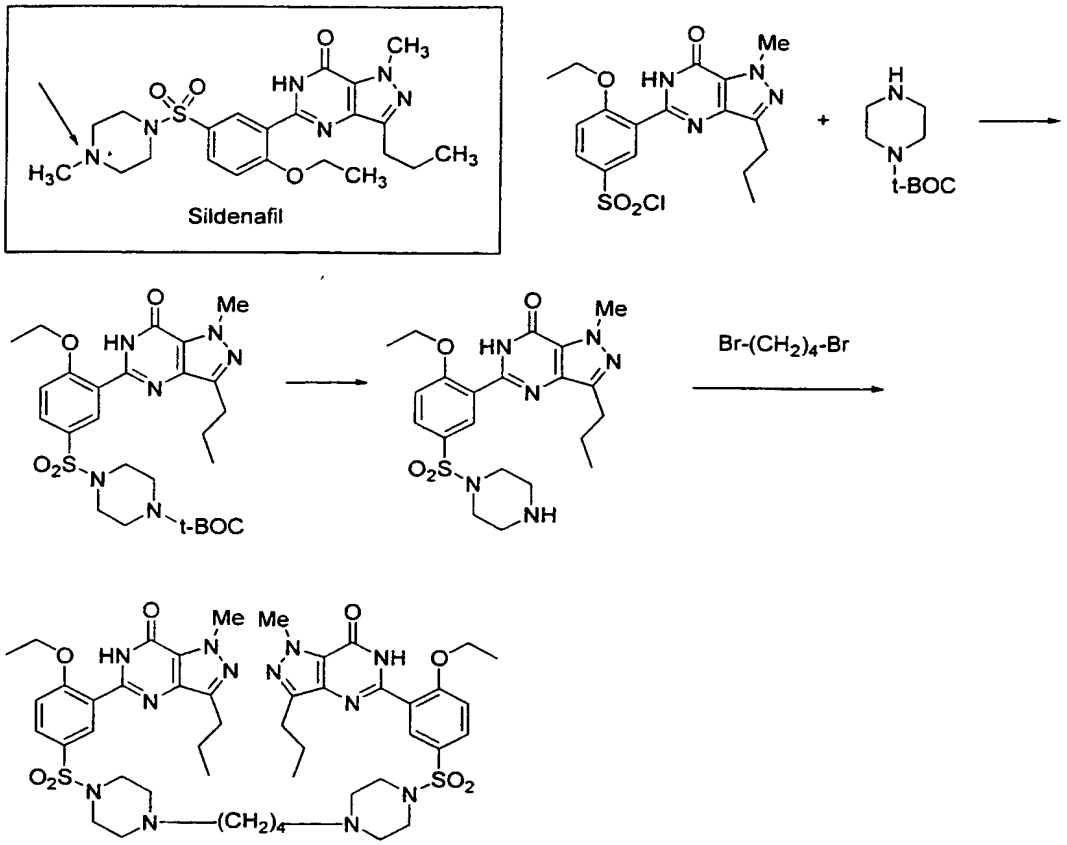
A reaction flask is charged with 20 mmols of 4-methoxy-pteridin-2-ylamine, reported in Roth, B. *et al.* J. Am. Chem. Soc., 1951, 73, 2969, and 25 mmols of [4-(2-amino-ethoxy)-phenyl]-thiourea, and 20 mL of dioxane added. The flask is equipped for distillation and heated to afford a slow rate. The reaction is followed by TLC and fresh dioxane as needed. When judged complete, the solvent is removed to afford the thioureido intermediate which is purified as necessary by crystallization or chromatography. A solution of 10 mmols of this intermediate in 5 mL of acetonitrile is treated with 12 mmols of iodoethane. When complete, the mixture is concentrated and partitioned between ethyl acetate and sat. sodium bicarbonate. The organic phase is washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

EXAMPLE 33



A solution of 2-[4-(2-aminoethoxy)phenoxy]ethylamine (5 mmol) in CH_2Cl_2 (20 mL) is added over a period of 6 hours to a solution of the sulfonyl chloride, prepared as described in European Patent 463756, (10 mmol) in CH_2Cl_2 (20 mL) and diisopropylethylamine (20 mmol). The progress of the reaction is monitored by TLC. When it is complete, the solution is washed with dilute $NaHCO_3$, then dried and evaporated. The residue is chromatographed to afford the desired product.

EXAMPLE 34



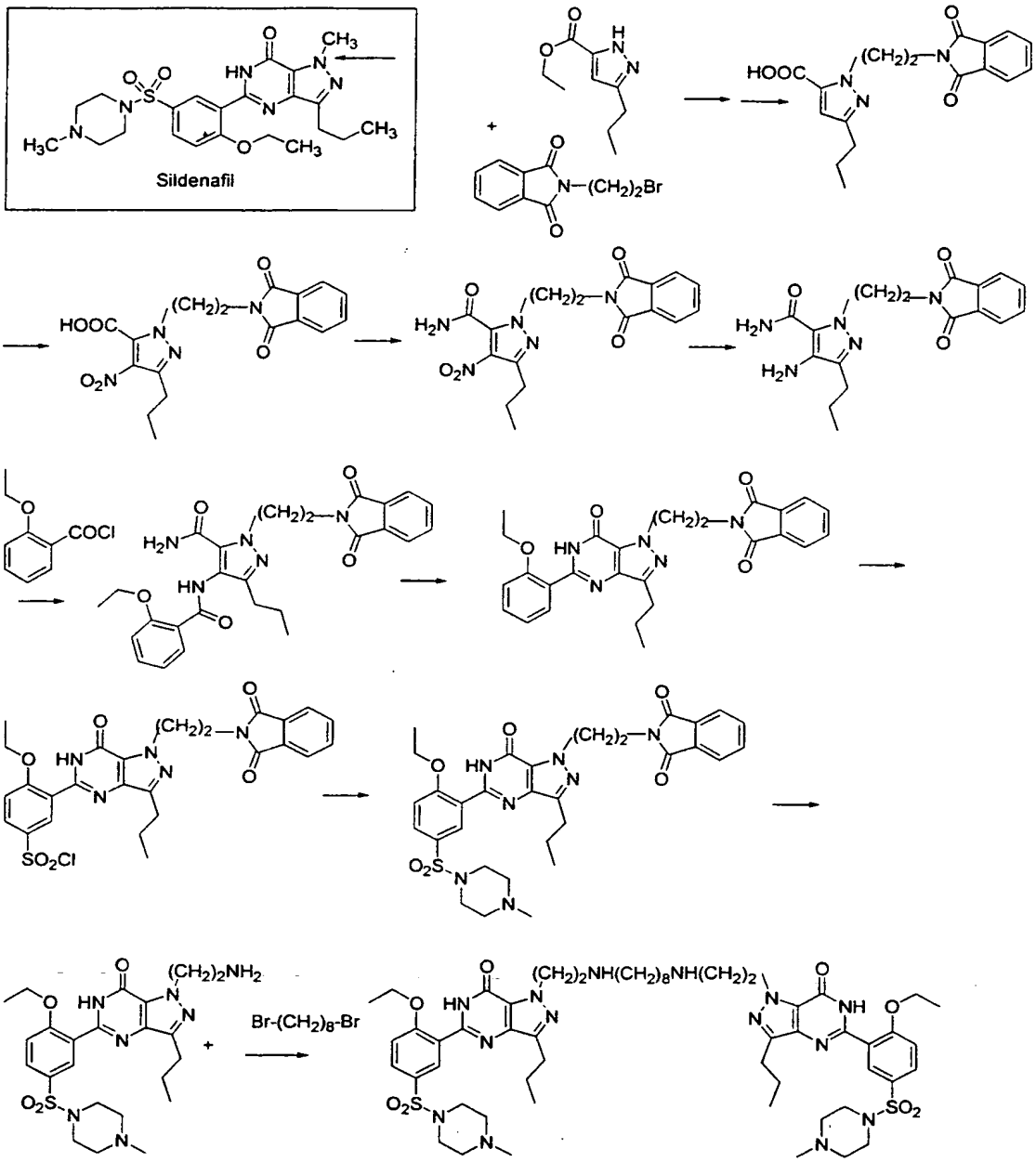
4-Tert-butoxycarbonylpiperazine (5 mmol) is added to a suspension of 5-[2-ethoxy-5-chlorosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, prepared as described in European Patent 463756, (2 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford 5-[2-ethoxy-5-(4-tert-butoxycarbonylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

The above compound (2 mmol) is dissolved in CH_2Cl_2 (25 mL) and TFA (3 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solvents are removed under vacuum, and the residue is dissolved in CH_2Cl_2 (50 mL); the solution is washed with dilute $NaHCO_3$, then dried and evaporated. The residue is chromatographed to afford the desired

piperazine.

1,4-Dibromobutane (10 mmol) and the piperazine (20 mmol) are heated at 60° in DMF (20 mL) containing K_2CO_3 (1 g) and KI (25 mg). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

EXAMPLE 35



Ethyl 3-propylpyrazole-5-carboxylate, prepared as described in Chem. Pharm. Bull., 1984, 32, 1568, (25 mmol) is dissolved in DMF (20 mL) containing K_2CO_3 (1 g) and KI (25 mg). 2-(Bromoethyl)phthalimide (30 mmol) is added, and the mixture is heated at 60°. The progress of the reaction is monitored by TLC. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is then dried and evaporated. The residue is chromatographed to afford ethyl 1-(2-phthalimidoethyl)-3-propylpyrazole-5-carboxylate.

The above ester (10 mmol) is dissolved in THF (20 mL) and a solution of LiOH (10 mmol) in water (20 mL) is added. The progress of the reaction is monitored by TLC. When it is complete, the reaction mixture is added to water, and washed with ether. The aqueous solution is acidified with dilute HCl, then extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 1-(2-phthalimidoethyl)-3-propylpyrazole-5-carboxylic acid.

The above compound (10 mmol) is added in portions to a mixture of oleum (5 mL) and fuming nitric acid (5 mL), at 60°. After 12 hours the mixture is cooled and added to ice. The mixture is extracted with EtOAc, and the extract is dried and evaporated under vacuum, The residue is chromatographed to afford 1-(2-phthalimidoethyl)-4-nitro-3-propylpyrazole-5-carboxylic acid.

The above acid (10 mmol) is dissolved in CH_2Cl_2 (20 mL) and $SOCl_2$ (20 mmol) and DMF (0.01 mL) are added. After 6 hours the volatile components are removed under vacuum. The residue is redissolved in CH_2Cl_2 (10 mL) and the solvent is again removed under vacuum, to afford the corresponding acid chloride. This compound (10 mmol) is dissolved in acetone, (10 mL) and the solution is added with vigorous stirring to concentrated NH_4OH (10 mL). After 1 hour the mixture is diluted with 3 volumes of water, and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 1-(2-phthalimidoethyl)-4-nitro-3-propylpyrazole-5-carboxamide.

The above compound (10 mmol) and $SnCl_2$ dihydrate (40 mmol) are suspended in EtOH (30 mL) and the mixture is heated at reflux. The progress of the reaction is monitored by TLC. When it is complete, the mixture is cooled and basified by addition of dilute NaOH. The solution is extracted with CH_2Cl_2, and the extract is dried and evaporated. The residue is chromatographed to afford 4-amino-1-(2-phthalimidoethyl)-3-propylpyrazole-5-carboxamide.

The above compound (50 mmol) is dissolved in CH_2Cl_2 (100 mL) and the solution is added to a solution of with 2-ethoxybenzoyl chloride (30 mmol), 4-dimethylaminopyridine (0.1 mmol) and Et_3N (100 mmol) in CH_2Cl_2 (100 mL) at 0°. The progress of the reaction is monitored by TLC. When it is complete, the reaction mixture is washed with dilute HCl, then dried and evaporated. The residue is chromatographed to afford 4-[(2-ethoxybenzamido]-1-(2-phthalimidoethyl)-3-propylpyrazole-5-carboxamide.

The above compound (25 mmol) is added in portions to a solution of NaOH (50 mmol) and

30% H_2O_2 (8 mL) in water (75 mL) and EtOH (10 mL). The mixture is heated at reflux, and the progress of the reaction is monitored by TLC. When it is complete, the reaction mixture is cooled and the solvents are removed under vacuum. To the residue is added dilute HCl (30 mL); the mixture is then extracted with CH_2Cl_2, and the extract is washed with aqueous Na_2CO_3, then is dried and evaporated. The residue is chromatographed to afford 5-[2-ethoxyphenyl)]-1-(2-phthalimidoethyl)-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

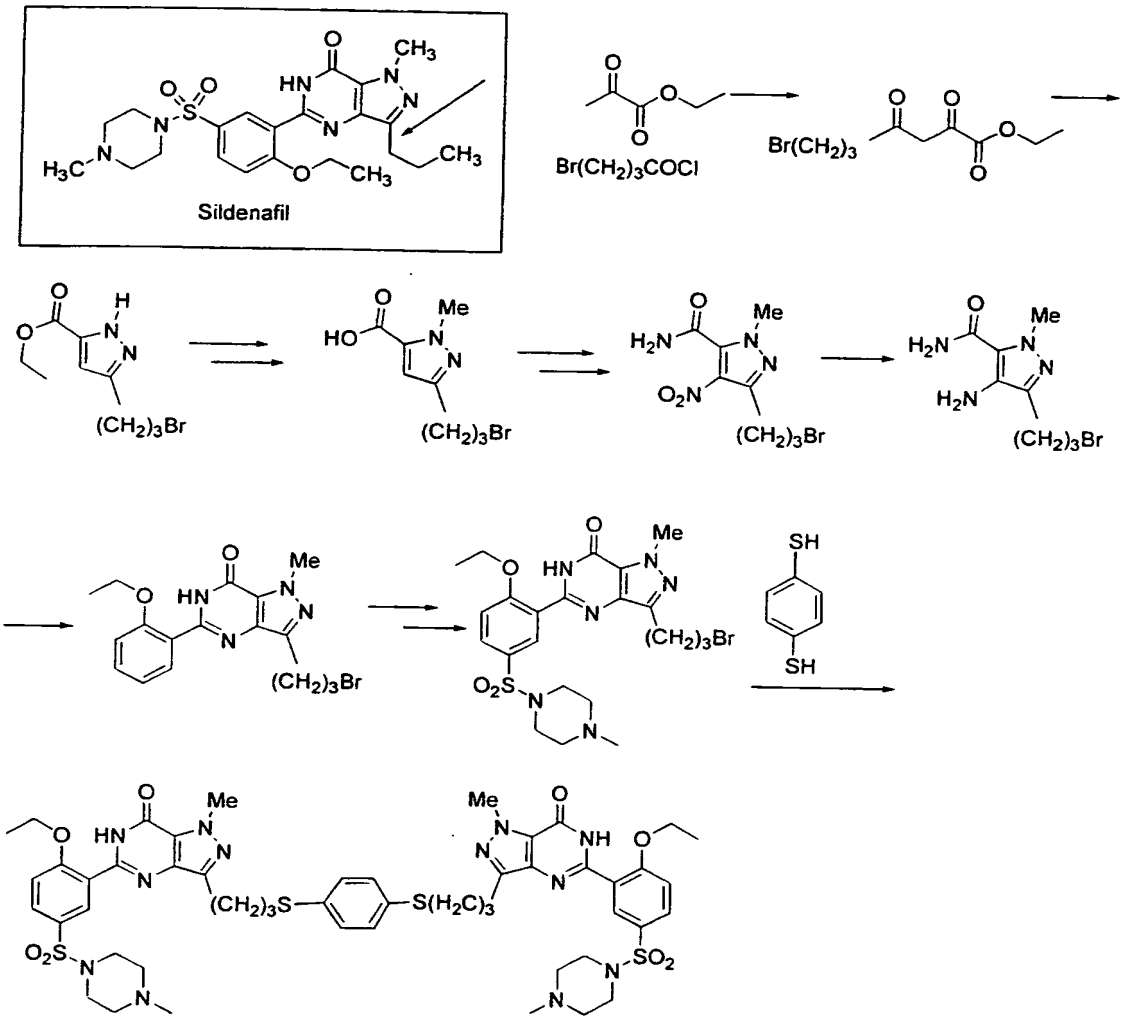
The above compound (10 mmol) is added to chlorosulfonic acid (10 mL) at 0° under nitrogen. The mixture is maintained at 0° for 12 hours, and is then added to water (100 mL). The solution is extracted with CH_2Cl_2, and the extract is dried and evaporated to afford 5-[2-ethoxy-5-chlorosulfonylphenyl)]-1-(2-phthalimidoethyl)-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

The above compound (5 mmol) is added to a suspension of N-methylpiperazine (2 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by TLC. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford 5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl)]-1-(2-phthalimidoethyl)-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

The above compound (10 mmol) is dissolved in EtOH (10 mL) and hydrazine hydrate (2 mL) is added. The progress of the reaction is monitored by TLC. When it is complete, the mixture is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford 1-(2-aminoethyl)-5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl)]-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

1,8-Dibromooctane (10 mmol) and the the bromoethyl compound prepared in the preceding step (20 mmol) are heated at 60° in DMF (20 mL) containing K_2CO_3 (1 g) and KI (25 mg). The progress of the reaction is monitored by TLC. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

EXAMPLE 36



Ethyl oxalate (0.1 mol) is dissolved in dry THF (100 mL) at 0° and sodium ethoxide (0.1 mol) is added in portions with stirring. After 1 hour, a solution of 4-bromobutyryl chloride, (0.1 mol) in dry THF (50 mL) is added. The mixture is stirred for 24 hours, and then is added to dilute HCl. The aqueous solution is extracted with ether, and the extract is dried and evaporated. The residue is chromatographed to afford the compound ethyl 7-bromo-2,3,5-trioxoheptanoate.

The above compound (0.1 mol) is dissolved in EtOH (100 mL) and 85% hydrazine hydrate (0.2 mol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH_2Cl_2; the extract is dried and evaporated and the residue is chromatographed to afford ethyl 3-(3-bromopropyl)pyrazole-5-carboxylate.

A mixture of the above compound (75 mmol) and dimethyl sulfate (75 mmol) is heated to 90° for 3 hours. The mixture is then cooled and dissolved in CH_2Cl_2. The solution is washed with dilute Na_2CO_3, and then dried and evaporated. The residue is chromatographed to afford ethyl 3-(3-bromopropyl)-1-methylpyrazole-5-carboxylate.

The above compound (0.1 mol) is dissolved in THF (200 mL) and a solution of LiOH (0.1 mol) in water (200 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the reaction mixture is added to water, and washed with ether. The aqueous solution is acidified with dilute HCl, then extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to the desired product.

The compound (10 mmol) is added in portions to a mixture of oleum (5 mL) and fuming nitric acid (5 mL), at 60°. After 12 hours the mixture is cooled and added to ice. The mixture is extracted with EtOAc, and the extract is dried and evaporated under vacuum, The residue is chromatographed to afford the desired product.

The compound (10 mmol) is dissolved in CH_2Cl_2 (20 mL) and $SOCl_2$ (20 mmol) and DMF (0.01 mL) are added. After 6 hours the volatile components are removed under vacuum. The residue is redissolved in CH_2Cl_2 (10 mL) and the solvent is again removed under vacuum, to afford the corresponding acid chloride. This compound (10 mmol) is dissolved in acetone, (10 mL) and the solution is added with vigorous stirring to concentrated NH_4OH (10 mL). After 1 hour the mixture is diluted with 3 volumes of water, and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the desired compound.

The compound (10 mmol) and $SnCl_2$ dihydrate (40 mmol) are suspended in EtOH (30 mL) and the mixture is heated at reflux. The progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and basified by addition of dilute NaOH. The solution is extracted with CH_2Cl_2, and the extract is dried and evaporated. The residue is chromatographed to 4-amino-3-(3-bromopropyl)-1-methylpyrazole-5-carboxamide.

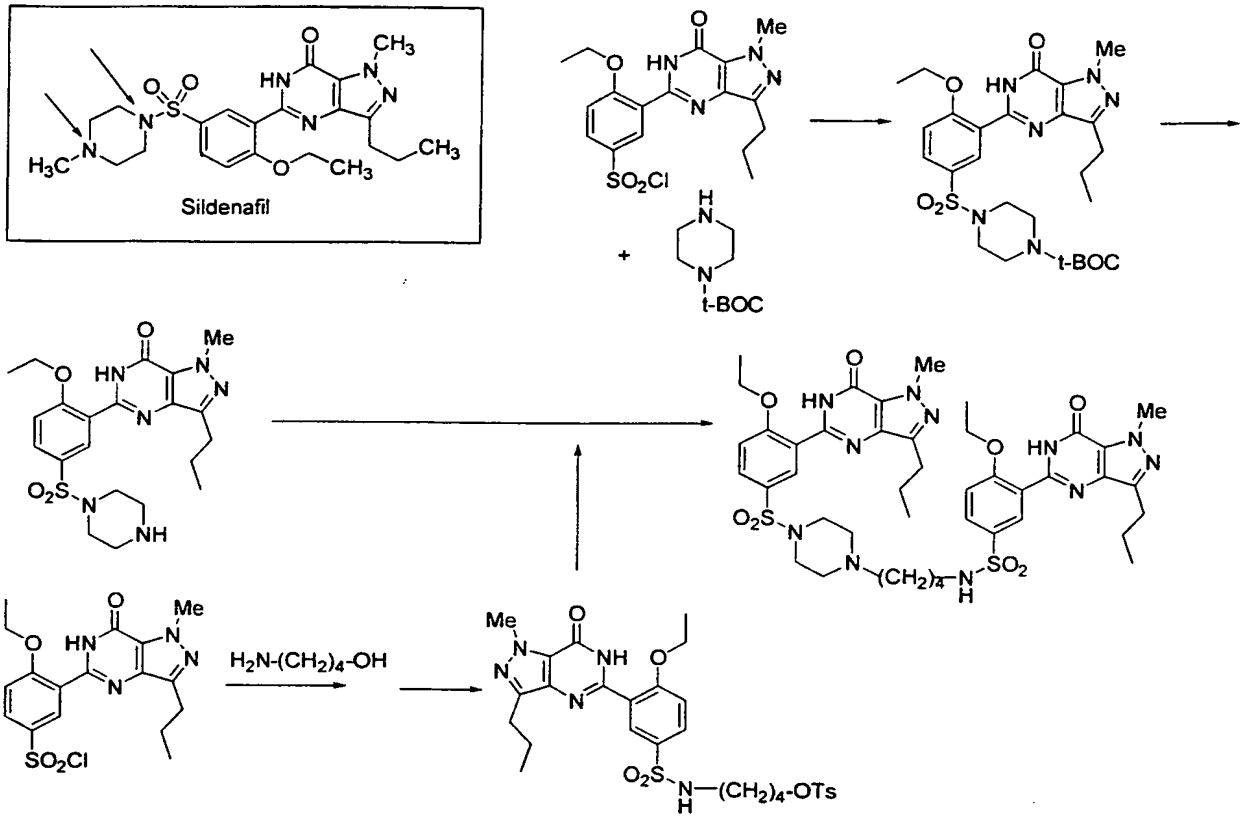
The above compound (50 mmol) is dissolved in CH_2Cl_2 (100 mL) and the solution is added to a solution of 2-ethoxybenzoyl chloride (30 mmol), 4-dimethylaminopyridine (0.1 mmol) and Et_3N (100 mmol) in CH_2Cl_2 (100 mL) at 0°. The progress of the reaction is monitored by tlc. When it is complete, the reaction mixture is washed with dilute HCl, then dried and evaporated. The residue is chromatographed to afford the desired product. The product thus obtained (25 mmol) is added in portions to a solution of NaOH (50 mmol) and 30% H_2O_2 (8 mL) in

water (75 mL) and EtOH (10 mL). The mixture is heated at reflux, and the progress of the reaction is monitored by tlc. When it is complete, the reaction mixture is cooled and the solvents are removed under vacuum. To the residue is added dilute HCl (30 mL); the mixture is then extracted with CH_2Cl_2, and the extract is washed with aqueous Na_2CO_3, then is dried and evaporated. The residue is chromatographed to afford 3-(3-bromopropyl)-5-[2-ethoxyphenyl)]-1-methyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

The above compound (10 mmol) is added to chlorosulfonic acid (10 mL) at 0° under nitrogen. The mixture is maintained at 0° for 12 hours, and is then added to water (100 mL). The solution is extracted with CH_2Cl_2, and the extract is dried and evaporated to afford the corresponding 5-chlorosulfonyl compound. N-Methyl piperazine (5 mmol) is added to a suspension of the above compound (2 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford the compound 3-(3-bromopropyl)-5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

A solution of 1,4-dimercaptobenzene (5 mmol) in THF (25 mmol) is added to a solution of the bromo compound (10 mmol) in THF (25 mL) and diisopropylethylamine (1 mL) at -20° under nitrogen. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the compound.

EXAMPLE 37



4-(Tert-butoxycarbonylpiperazine (5 mmol) is added to a suspension of 5-[2-ethoxy-5-chlorosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, prepared as described in European Patent 463756, (2 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford 5-[2-ethoxy-5-(4-tert-butoxycarbonylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

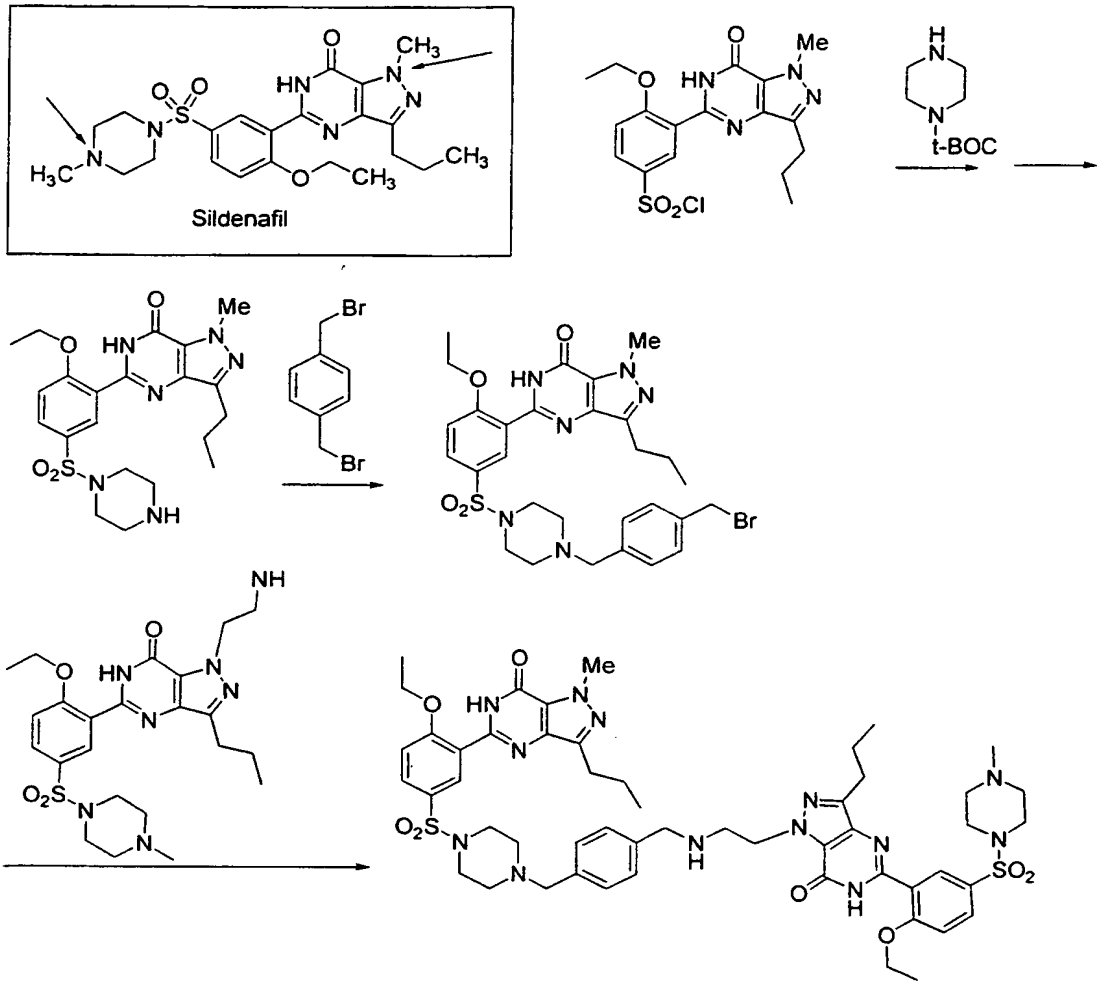
The above compound (2 mmol) is dissolved in CH_2Cl_2 (25 mL) and TFA (3 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solvents are removed under vacuum, and the residue is dissolved in CH_2Cl_2 (50 mL); the solution is washed with dilute $NaHCO_3$, then dried and evaporated. The residue is chromatographed to afford the piperazine.

The sulfonyl chloride (5 mmol), prepared as described in EP463756, is added to a suspension of 4-aminobutanol (5 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford the desired compound.

This compound (1 mmol) is dissolved in pyridine (10 mL) and p-toluenesulfonyl chloride (1.1 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH_2Cl_2; the extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

One molar equivalent of this compound is dissolved in DMF (20 mL) containing K_2CO_3 (1 g) and KI (25 mg). One molar equivalent of the amine is added and the mixture is heated at 60°. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is then dried and evaporated. The residue is chromatographed to afford the desired compound.

EXAMPLE 38



4-(Tert-butoxycarbonylpiperazine (5 mmol) is added to a suspension of 5-[2-ethoxy-5-chlorosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, prepared as described in European Patent 463756, (2 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford 5-[2-ethoxy-5-(4-tert-butoxycarbonylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one.

The above compound (2 mmol) is dissolved in CH_2Cl_2 (25 mL) and TFA (3 mL) is added.

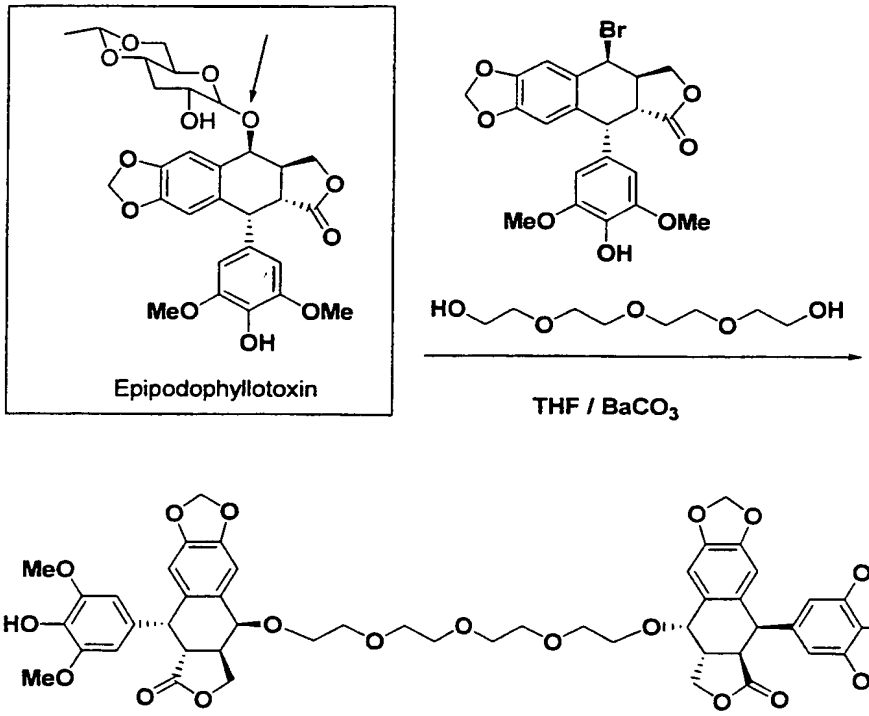
The progress of the reaction is monitored by tlc. When it is complete, the solvents are removed under vacuum, and the residue is dissolved in CH_2Cl_2 (50 mL); the solution is washed with dilute $NaHCO_3$, then dried and evaporated. The residue is chromatographed to afford the desired piperazine.

The above amine, (5 mmol) is added to a suspension of 1,4-di(bromomethyl)benzene (5 mmol) in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford the desired compound.

This compound is then reacted as described above, with the amine (synthesis is described following this step), to afford the desired compound.

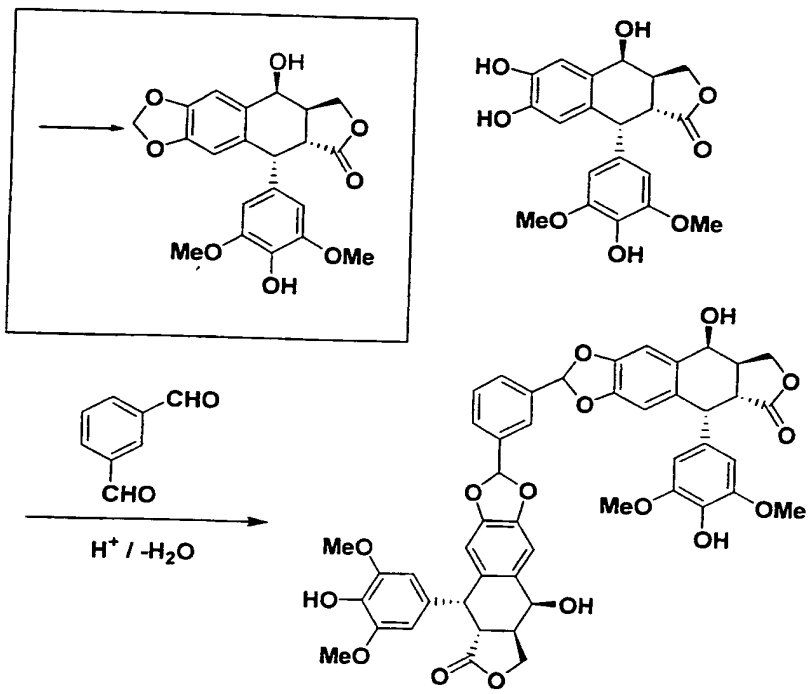
The amine prepared in Example 35 (5 mmol) is added to a suspension of the bromide (5 mmol) prepared above in EtOH (50 mL). The mixture is stirred at room temperature, and the progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under vacuum, and the residue is redissolved in 9:1 CH_2Cl_2:MeOH (100 mL); the solution is washed with aqueous Na_2CO_3, then dried and evaporated. The residue is chromatographed to afford the desired compound.

EXAMPLE 39



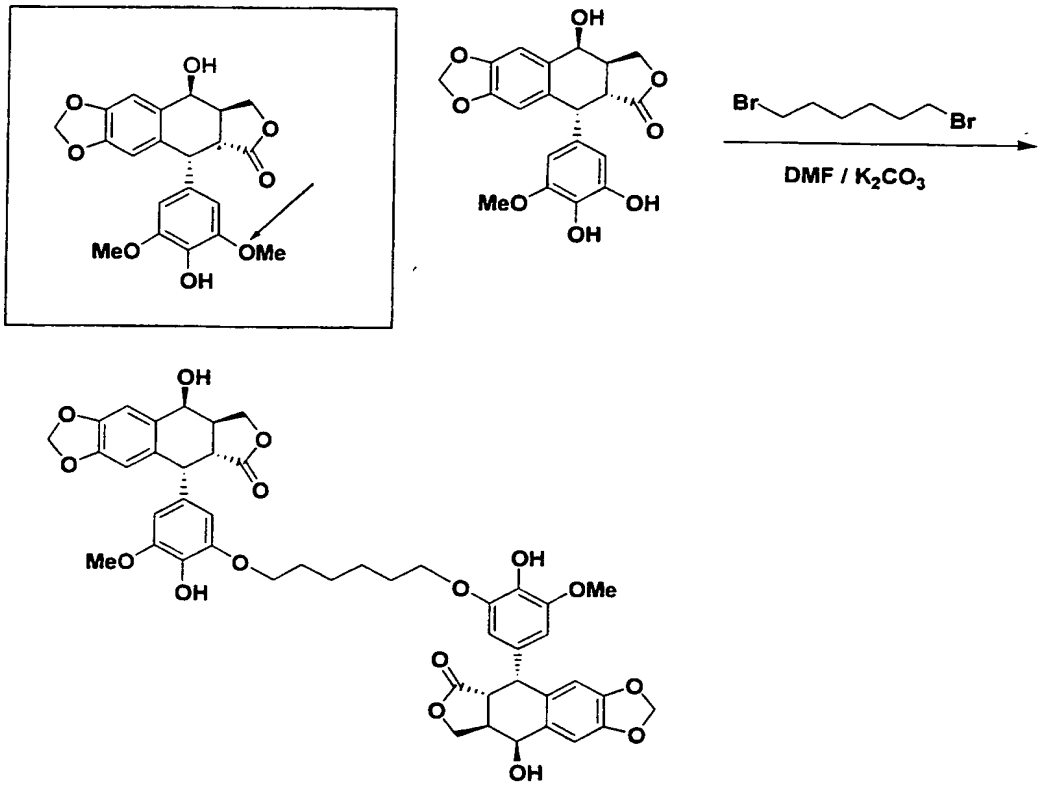
A solution of 1.2 mmols of the alkyl bromide, reported in Thurston, Lee S., *et al.* J. Med. Chem., 1996, **29**, 1547, in 15 mL of anhyd. THF is treated with 2.4 mmols of anhyd. barium carbonate and 0.6 mmols of tetraethylene glycol and kept overnight at room temperature. After filtering and concentrating, the residue is purified by chromatography to afford the desired compound.

EXAMPLE 40



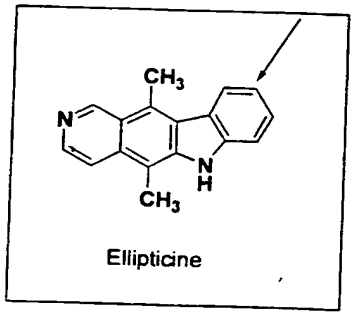
A solution of 20 mmols of the starting compound, which is described in Long, Byron H., Casazza, Anna-Maria Cancer Chemother. Pharmacol., 1994, **34**(Suppl.), S26, in 50 mL of toluene with 10 mmols of isophthalaldehyde and 100 mg of p-TSA is refluxed with the azeotropic removal of water. The reaction is followed by TLC and when judged complete, is cooled and washed with sat. sodium bicarbonate, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

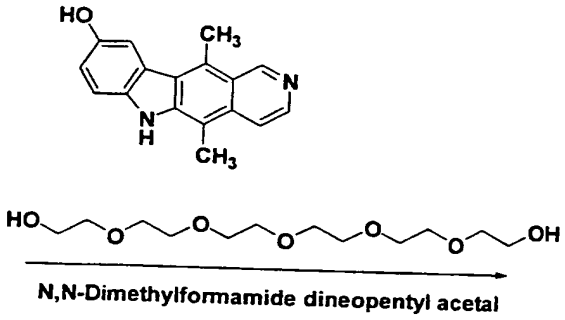
EXAMPLE 41

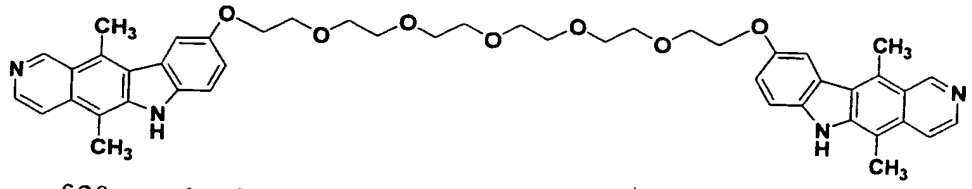


A solution of 50 mmols of the starting compound, described in Lee, Kuo-Hsiung, *et al.* J. Nat. Prod., 1992, **55**, 1100, in 20 mL of DMF with 25 mmols of 1,6-dibromohexane and 50 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

EXAMPLE 42

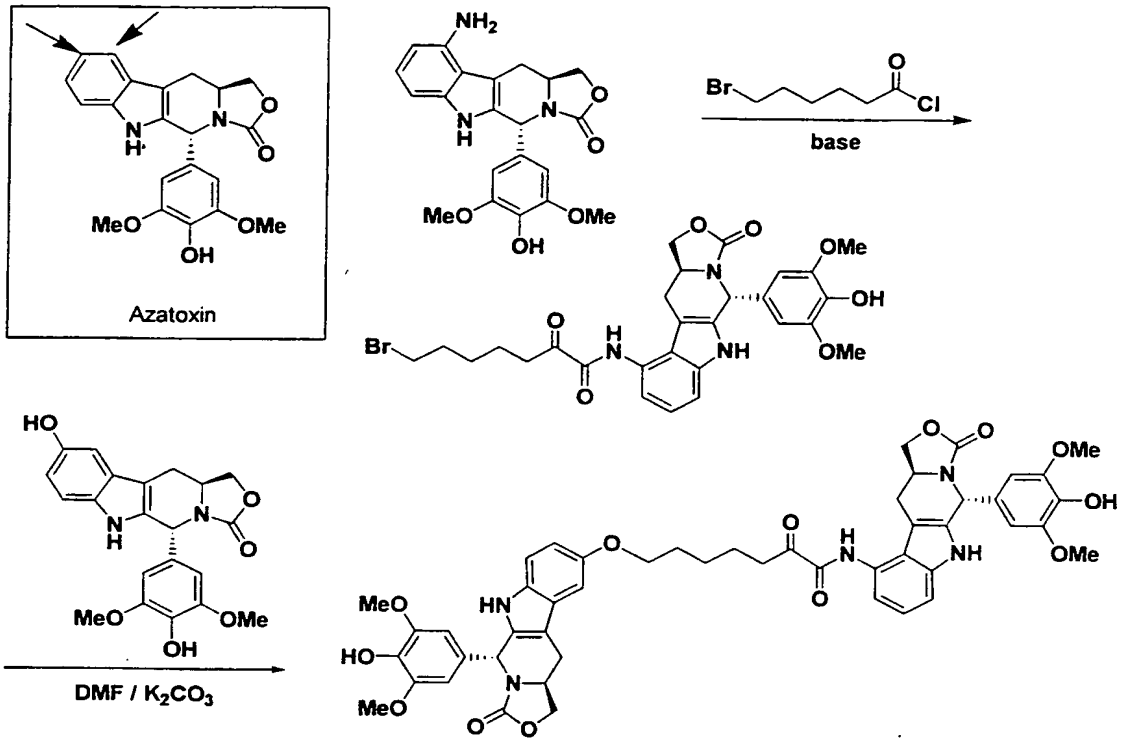






A mixture of 20 mmols of the starting compound, which is described in Guthrie, Robert W., *et al.* J. Med. Chem., 1975, 18, 755, 10 mmols of hexaethylene glycol and 4 mL of N,N-dimethylformamide dineopentyl acetal is kept at 115-120 °C for 24hr. After cooling, the reaction is diluted with ethyl acetate, washed with water, dried over sodium sulfate and the solvent removed. The residue is purified by chromatography to afford the desired product.

EXAMPLE 43



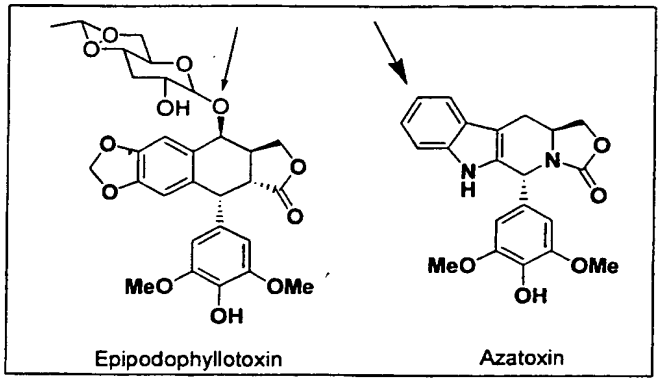
[Preparation of the aniline starting material: To a solution of 68.1 mmol of sodium ethoxide in 150 mL of absolute ethanol is added 68.02 mmol of L-5-hydroxytryptophanol in 100 mL of ethanol and 74.8 mmols of diethyl carbonate. The solution is refluxed for 5 hr. then concentrated. The residue is partitioned between sat. NH_4Cl and methylene chloride and the aqueous phase again extracted with methylene chloride. The combined organic phases are dried over sodium sulfate and the solvent removed. The product is purified by chromatography and/or crystallization. To a solution of 2 mmols of the above oxazolidinone intermediate in 8 mL of anhyd. THF with 3 mmols of syringaldehyde dimethyl acetal is added 0.2 mmols of anhyd. trifluoroacetic acid and the solution heated to reflux. The reaction is followed by TLC and when judged complete partitioned between sat. sodium bicarbonate and methylene chloride. The organic phase is dried over sodium sulfate and the solvent removed. The residue is purified by crystallization or chromatography to afford the desired compound].

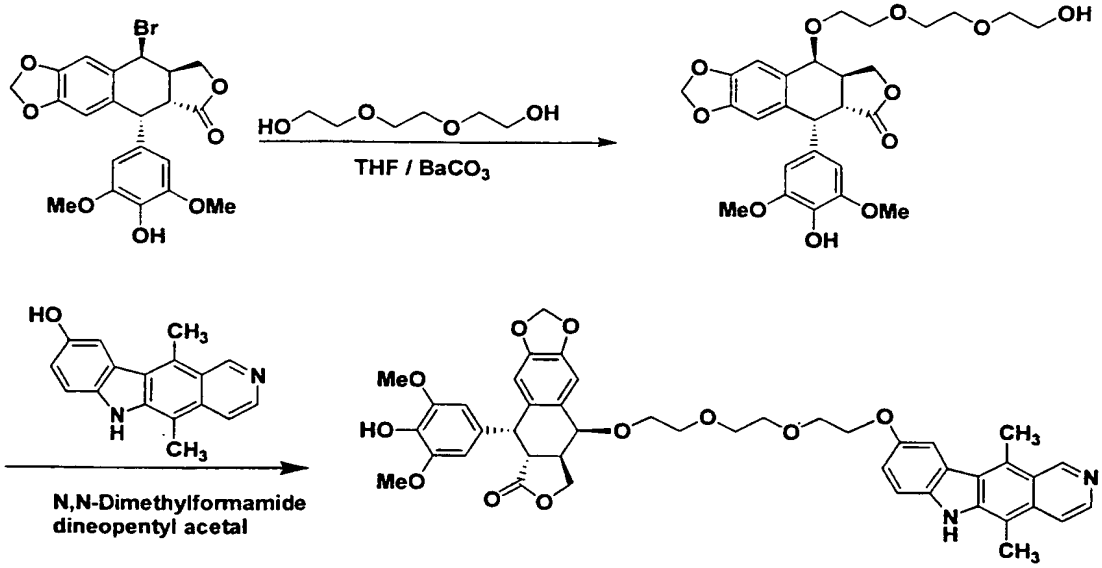
A solution of 20 mmols of this amine in 50 mL of ethyl acetate with 20 mmols of triethylamine is treated with 20 mmols of 6-bromohexanoyl chloride at room temperature. After 1hr., the reaction is washed with water, dried over sodium sulfate and the solvent removed *in*

vacuo. The residue is purified by chromatography to afford the desired product.

A solution of 10 mmols of this compound, 10 mmols of the phenol shown (prepared in a manner analagous to that of the aniline starting material) and 10 mmols of potassium carbonate in 10 mL of DMF is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

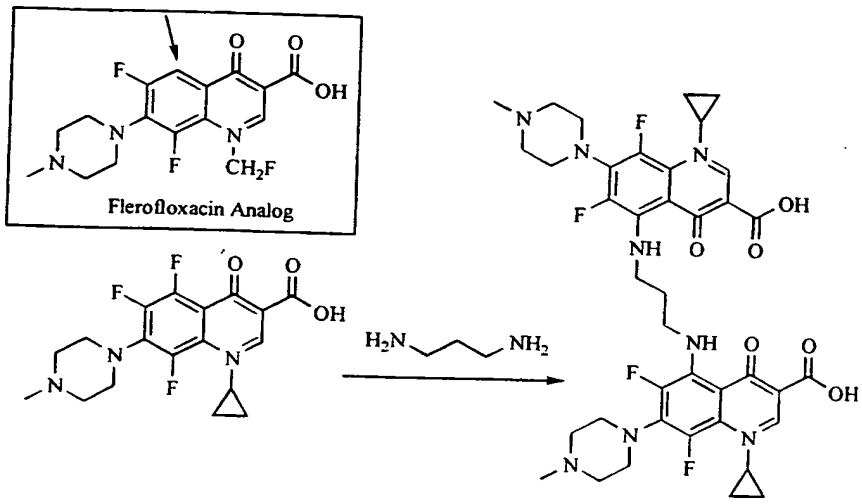
EXAMPLE 44





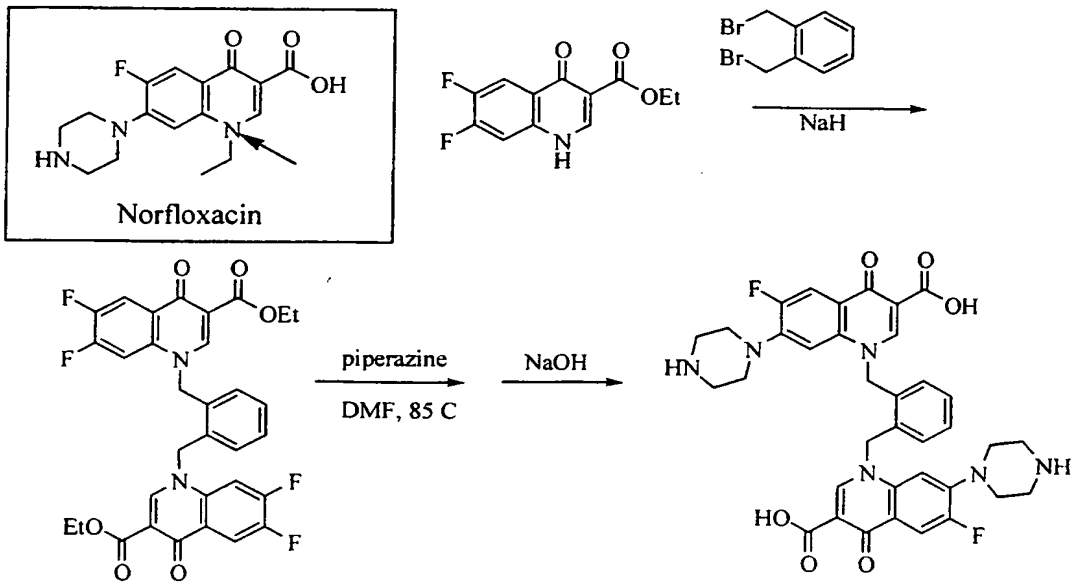
To a well stirred mixture of 40 mmols of anhyd. barium carbonate in 100mL anhyd. THF with 100 mmols of triethlyene glycol is slowly added 20 mmols of the alkyl bromide, reported in Thurston, Lee S., *et al.* J. Med. Chem., 1996, 29, 1547. After 24 hr., the mixture is concentrated and purified by chromatography. A mixture of 20 mmols of this intermediate, 15 mmols of the phenol shown (described in Guthrie, Robert W., *et al.* J. Med. Chem., 1975, 18, 755), and 4 mL of N,N-dimethylformamide dineopentyl acetal is kept at 115-120 C for 24hr. After cooling, the reaction is diluted with ethyl acetate, washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired compound.

EXAMPLE 45



1-Cyclopropyl-5,6,8-trifluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0. 3g, 0.8 mmol), prepared according to the procedure in J Med Chem 1989, 32, 1313,was mixed with 0.033 mL (0.4 mmol) of 1,3-diaminopropane and 0.55 mL (3.2 mmol) of N,N-diisopropylethylamine in 4 mL of DMPU, and the mixture was heated at 85°C for 18 hours. The reaction mixture was cooled and precipitated from 30 mL of 2:1 Hexane/Ether. The crude precipitate was taken up in 5:1 0.5N HCl: acetonitrile and purified by reverse phase HPLC using a 10-60% acetonitrile (0.1% TFA) gradient over 60 min. Product peaks were determined by MS and purity analyzed by HPLC. The desired product fractions were combined and lyophilized to obtain a white fluffy solid. This material was then taken up in 40 mL of 0.25M HCl and lyophilized twice to yield 0.14 g (0.14 mmol, 17%) of N,N'-bis-(1-cyclopropyl-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinolin -3-carboxylic acid-5-yl)-1,3-diaminopropane as a dark yellow solid.

EXAMPLE 46



0.3 g (1.2 mmol) of 6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester, prepared as described in J. Med. Chem. 1996, 39, 436-445, was mixed with 7 mL of DMF, 0.052 g (1.32 mmol) of 60% NaH was added, and the mixture was stirred under N_2 for 10 minutes at room temperature and then 3 minutes at 80°C, to produce a clear solution. The solution was cooled to room temperature and 0.14 g (0.54 mmol) of 1,2-bisbenzyl bromide was added. The mixture was stirred at 85°C for 18 hours. The reaction was then cooled and precipitated from a 2:1 mixture of diethyl ether and ethyl acetate. The resulting solid was collected by filtration and washed with ether to obtain 0.42 g of 1,2-bis-(6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester-1-meth-1-yl)-benzene, which was used directly in the next reaction.

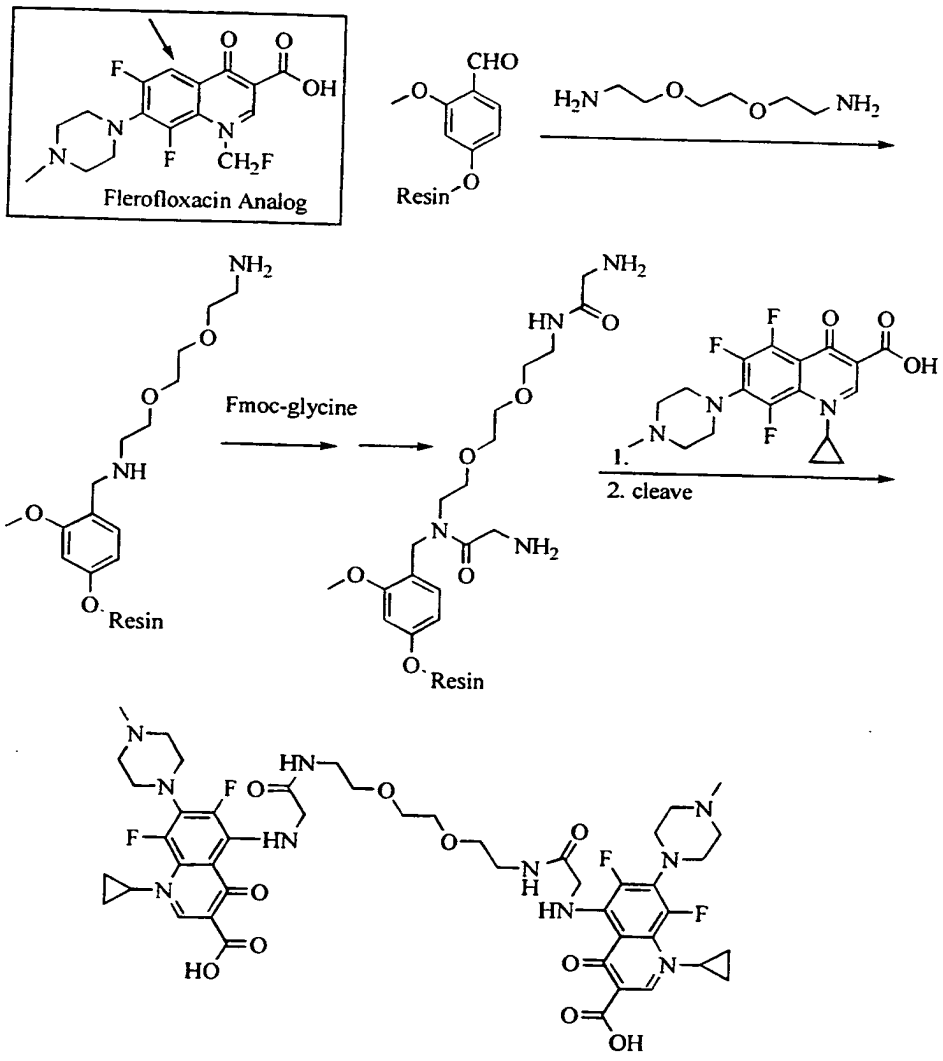
0.16 g (0.3 mmol) of the crude product from above was mixed with 0.068 g (0.9 mmol) of piperazine in 3 mL of DMF. The mixture was heated in an 85°C oil bath for 2 hours. The mixture was cooled and poured into ethyl acetate. The solid was collected, washed with ether, and dried to obtain 0.15 g of crude 1,2-bis-(6-fluoro-7-(piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester-1-yl)- 1,2-dimethylbenzene, which was used directly in the next reaction.

0.05 g of the crude product from above was mixed with 5 mL of 2N NaOH and heated for 1.5 hours in a 100°C oil bath. The reaction was then cooled and acidified with conc. HCl. 2 mL of acetonitrile was added to solubilize the precipitate and the solution was purified by prep.

HPLC using a 5-40% acetonitrile/0.1%aqueous TFA gradient over 40 min. Pure product fractions were determined by LC/MS and were lyophilized to yield 19 mgs (0.02 mmol) of crude 1,2-bis-(6-fluoro-7-(piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid-1-yl)-1,2-dimethylbenzene as the bis TFA salt.

5

EXAMPLE 47



A capsule of Argogel MBCHO resin (1 cap=0.157g; 0.35-0.45 mmol/g) was suspended in 3 mL of 1,2-dichoroethane in a 5 mL reaction vessel in an Argonaut Quest 210 Organic Synthesizer. The resin was agitated for 5 minutes and the solvent drained. Sodium

10

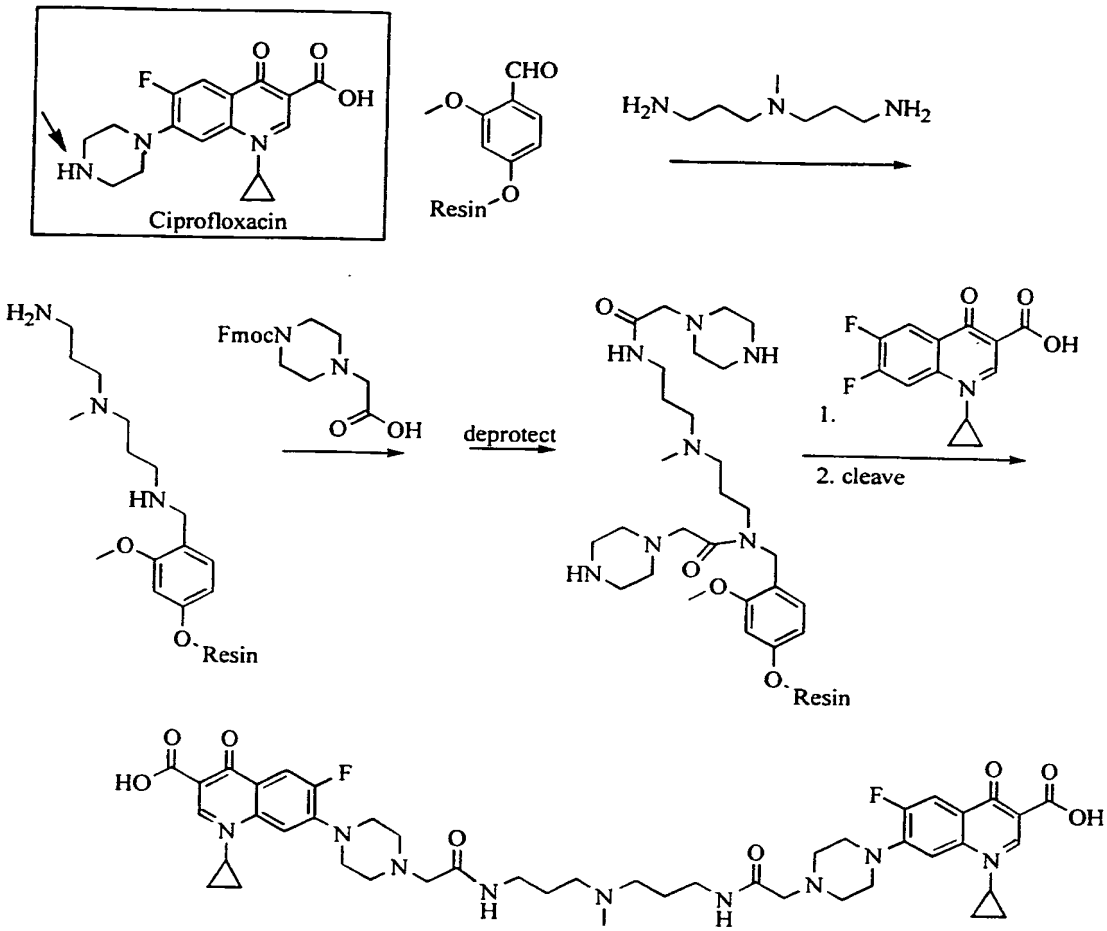
triacetoxyborohydride (0.46 mmol; 7eq based on resin) was weighed into each of the reaction vessels and 3 mL of dichloroethane was added and the resulting mixture agitated for 5 minutes. 2,2'-(Ethylenedioxy)bis(ethylamine) (5 equivalent based on resin) was added, and. the resin was agitated overnight at room temperature. The solvent and reactants were washed away by successive washings of methanol (x3), dichloromethane (x3) and 10% DIPEA in dichloromethane (x1). Prior to the acylation of step 2 the resin was washed once with N-methylpyrrolidine (NMP).

Fmoc-glycine (0.39 mmol; 6 equivalent based on resin) was dissolved in 3 mL of NMP. HOAT (0.19 mmol; 3 equivalent based on resin) and HATU (0.2 mmol; 3 equivalent based on resin) with diisopropylethylamine (0.47 mmol; 7.2 equivalent based on resin). The solutions were activated for 5 minutes before addition to the diamine-substituted resin, and then added in equal portions to each reaction well. The resin mixture was agitated for 3 hours at room temperature. The resin was washed with NMP (x3) and DMF (x3). The Fmoc group was removed with 20% piperidine in DMF (2x15 minute). The piperidine was washed away with DMF (x3), methanol (x3) and dichloromethane (x3). Prior to acylation with the quinolone the resin was washed with NMP (x1).

1-Cyclopropyl-7-(4-methylpiperazine)-5,6,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.4 mmol; 6 equivalent based on resin) was dissolved in 3 mL of DMPU and added to the above product. Disopropylethylamine (1.55 mmol; 4 equivalent based on quinolone) was added and the resin mixture shaken at 85°C overnight. The excess quinolone was washed away with successive washings of DMPU (x1), DMF (x3), methanol (x3) and DCM (x3). The resin was completely dried by passing a stream on nitrogen gas through the reaction vessels for 2 hours prior to cleavage.

Cleavage from the resin was performed on all vessels using 5 mL of 95% TFA/5% water for 5 hours at 50°C. The cleavage solution was then drained off into 40 mL EPA vials and the resin was washed with an additional 3 mL of TFA. The TFA was removed using a Savant SpeedVac evaporator to yield the crude product (see Table 2). The compounds were then subjected to preparatory LC/MS purification. Analytical HPLC and MS confirmed the identity of the collected fractions to afford the desired compound.

EXAMPLE 48



A capsule of Argogel MBCHO resin (1 cap=0.157g; 0.35-0.45 mmol/g) was suspended in 3 mL of 1,2-dichoroethane in a 5 mL reaction vessel. The resin was agitated for 5 minutes and the solvent drained. Sodium triacetoxyborohydride (0.46 mmol; 7eq based on resin) and 3mL of dichloromethane was added into each of the reaction vessels and agitated for 5 minutes. 3,3'-Diamino-N-methyldipropylamine (5 equivalent based on resin) was added and the resin was agitated overnight at room temperature. The solvent and reactants were washed away by successive washings of methanol (x3), dichloromethane (x3) and 10% DIPEA in dichloromethane (x1). Prior to performing the next step, the resin was washed once with NMP.

4-Fmoc-piperazin-1-ylacetic acid hydrate (0.39 mmol; 6 equivalent based on the resin substitution) was dissolved in 3 mL of NMP. HOAT (0.19 mmol; 3 equivalent based on resin) and HATU (0.2 mmol; 3 equivalent based on resin) were added along with DIPEA (0.47 mmol; 7.2 equivalent based on resin). The solution was activated for 5 minutes before addition to the

diamine-substituted resin obtained in step 2). The mixture was agitated for 3 hours at room temperature, and then the acid was washed away with NMP (x3) and DMF (x3). The Fmoc group was removed with 20% piperidine in DMF. The piperidine was washed away with DMF (x3), methanol (x3) and DCM (x3). Prior to performing the next step, the resin was washed with NMP (x1).

1-Cyclopropyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4-methoxybenyl) ester (0.4 mmol; 6 equivalent based on resin substitution) was suspended in 3 mL of DMPU and added to the resin. DIPEA (1.55 mmol; 4 equivalent based on quinolone) was added and the resin mixture shaken at 115°C overnight. The excess quinolone was washed away with successive washings of DMPU (x1), DMF (x3), methanol (x3) and DCM (x3). The resin was completely dried by passing a stream on nitrogen gas through the reaction vessels for 2 hours prior to cleavage.

Cleavage was performed on all vessels using 5 mL of 95% TFA/5% water for 5 hours at 50°C. The cleavage solution was then drained off into 40 mL EPA vials and the resin was washed with an additional 3 mL of TFA. The TFA was removed using a Savant Speedvac evaporator. The compounds were then subjected to preparatory LC/MS purification using acetonitrile/water with 0.1 % TFA gradients. Collected fractions were concentrated using a SpeedVac. Analytical HPLC and MS confirmed the identity of the collected fractions.

EXAMPLE 49

This example illustrates the preparation of a representative pharmaceutical formulation for oral administration containing an active compound of Formula I.

Ingredients	Quantity per tablet, mgs.
Active Compound	200
Lactose, spray-dried	148
Magnesium stearate	2

The above ingredients are mixed and introduced into a hard-shell gelatin capsule. Other compounds of Formula I can be used as the active compound in the preparation of the orally administrable formulations of this example.

EXAMPLE 50

This example illustrates the preparation of another representative pharmaceutical formulation for oral administration containing a compound of Formula I.

Ingredients	Quantity per tablet, mgs.
Active Compound	400
Cornstarch	50
Lactose	145
Magnesium stearate	5

The above ingredients are mixed intimately and pressed into single scored tablets. Other compounds of Formula I can be used as the active compound in the preparation of the orally administrable formulations of this example.

EXAMPLE 51

This example illustrates the preparation of a representative pharmaceutical formulation containing a compound of Formula I.

An oral suspension is prepared having the following composition.

Ingredients	
Active Compound	1.0 g
Fumaric acid	0.5 g
Sodium chloride	2.0 g
Methyl paraben	0.1 g
Granulated sugar	25.5 g
Sorbitol (70% solution)	12.85 g
Veegum K (Vanderbilt Co.)	1.0 g
Flavoring	0.035 ml
Colorings	0.5 mg
Distilled water	q.s. to 100 ml

Other compounds of Formula I can be used as the active compound in the preparation of the orally administrable formulations of this example.

EXAMPLE 52

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula I.

An injectable preparation buffered to a pH of 4 is prepared having the following composition:

Ingredients	
Active Compound	0.2 g
Sodium Acetate Buffer Solution (0.4 M)	2.0 ml
HCL (1N)	q.s. to pH 4
Water (distilled, sterile)	q.s. to 20 ml

Other compounds of Formula I can be used as the active compound in the preparation of the injectable formulations of this example.

EXAMPLE 53

This example illustrates the preparation of a representative pharmaceutical formulation for injection containing an active compound of Formula I.

A reconstituted solution is prepared by adding 20 ml of sterile water to 1g of the compound of Formula I. Before use, the solution is then diluted with 200 ml of an intravenous fluid that is compatible with the compound of Formula I. Such fluids are chosen from 5% dextrose solution, 0.9% sodium chloride, or a mixture of 5% dextrose and 0.9% sodium chloride. Other examples are lactated Ringer's injection, lactated Ringer's plus 5% dextrose injection, Normosol-M and 5% dextrose, isolyte E, and acylated Ringer's injection

Other compounds of Formula I can be used as the active compound in the preparation of the injectable formulations of this example.

EXAMPLE 54

This example illustrates the preparation of a representative pharmaceutical formulation for topical application containing a compound of Formula I.

Ingredients	grams
Active compound	0.2-10
Span 60	2
Tween 60	2
Mineral oil	5
Petrolatum	10
Methyl paraben	0.15
Propyl paraben	0.05
BHA (butylated hydroxy anisole)	0.01
Water q.s. to 100	

All of the above ingredients, except water, are combined and heated to 60°C with stirring. A

sufficient quantity of water at 60°C is then added with vigorous stirring to emulsify the ingredients, and water then added q.s. 100 g.

Other compounds of Formula I can be used as the active compound in the preparation of topical formulations of this example.

EXAMPLE 55

This example illustrates the preparation of a representative pharmaceutical formulation containing a compound of Formula I.

A suppository totalling 2.5 grams is prepared having the following composition:

Ingredients	
Active Compound	500 mg
Witepsol H-15*	balance

(*triglycerides of saturated vegetable fatty acid; a product of Riches-Nelson, Inc., New York, N.Y.)

Other compounds of Formula I can be used as the active compound in the preparation of the suppository formulations of this example.

EXAMPLE 56
In Vivo Cholesterol Biosynthesis Inhibition in Rats

The methods used for intravenous (iv) and oral (po) drug testing can be adapted from a procedure originally described in U.S. Patent No. 4,613,610 and PCT Int. Application WO 86/00367, the disclosures of which are incorporated herein by reference in their entirety. Male Sprague-Dawley rates (200-300 g) are adapted to a reverse light cycle for 7-10 days and fed Purina rat chow (no. 5001) ad libitum. In order to measure cholesterol synthesis, sodium [1-^{14}C] acetate (1-3 mCi/mmol) (25 □Ci/100 g of body weight) is injected intraperitoneally (ip) 2 h before the mid-dark point in the diurnal cycle. Two hours after the mid-dark point animals are anesthetized ip with ketamine/xylazine and bled into EDTA-treated centrifuge tubes from the abdominal aorta. Plasma is obtained by centrifugation at 1100g for 10 min. One-milliliter plasma samples are aliquoted and either processed directly or frozen at -20□C. For iv testing,

the salt forms of test compounds are routinely dissolved in saline and injected iv into the tail vein 5 min before [^{14}C] acetate injection. For po testing, drugs are dissolved in saline and given by gavage 30 min before [^{14}C] acetate injection. Cholesterol synthesis is measured by determining the level of ^{14}C-labeled nonsaponifiable lipid present in 1 mL of plasma; the method used is a modification of the method described by Dugan et al., Arch. Biochem. Biophys. 1972, 152, 21-27, the disclosure of which is incorporated herein by reference in its entirety. One milliliter physiological saline is added to 1 mL of plasma, followed by the addition of 5.0 mL of 10% KOH in absolute ethanol. Samples are mixed and saponified at 75□C for 1 h. After cooling, approximately 0.02 □Ci (44,000 dpm) [1,2-^{3}H] cholesterol (40-60 Ci/mmol.) is added to each sample. Samples are extracted once with 5 mL of petroleum ether, and the organic phase is backwashed with 5 mL of saline. This extraction procedure typically results in 50-90% recovery of the added [^{3}H] cholesterol internal standard. The extracts are dried in glass vials, and the residue resuspended in 0.5 mL of $CHCl_3$-MeOH (2:1). Samples are counted for both ^{3}H and ^{14}C in 10 mL of Optifluor scintillation fluid. The [^{3}H] cholesterol internal standard recovery value from each sample is used to correct each sample to 100% recovery of [^{14}C] cholesterol. In early experiments, sample extract residues are redissolved in 100 mL of $CHCl_3$-MeOH (2:1) and chromatographed on silica gel (Whatman LK6D) thin-layer plates using either hexanes-Et_2O-HOAc (75:25:1) or CH_2Cl_2-acetone (60:1). Using either chromatographic system, greater than 90% of the ^{14}C-label cochromatographed with authentic cholesterol. Thus, to simplify the method, the TLC step is omitted in subsequent experiments and results are calculated as ^{14}C-labeled nonsaponifiable plasma lipid values, of which, greater than 90% of the ^{14}C-label is authentic cholesterol. The percent inhibition of cholesterol synthesis is derived by comparing ^{14}C-labeled nonsaponifiable plasma lipid values per milliliter of plasma from control and drug-treated animal groups (4-5 rats/group). Percent inhibition is plotted relative to the log drug dose and a linear best fit regression line is determined for each experiment. Mean ED_{50} values (level of drug required to suppress cholesterol synthesis in vivo by 50%) are calculated from two or more experiments.

EXAMPLE 57

Rat Carragenan Foot Pad Edema Test

The carragenan foot edema test can be performed with materials, reagents and procedures essentially as described by Winter et al. (Proc. Soc. Exp. Biol. Med., 111, 544 (1962)). Male Sprague-Dawley rats are selected in each group so that the average body weight is as close as possible. Rats are fasted with free access to water for over sixteen hours prior to the test. The rats are dosed orally (1 mL) with the test compound suspended in vehicle containing 0.5% methylcellulose and 0.025% surfactant, or with vehicle alone. One hour later a subplantar injection of 0.1 mL of 1% solution of carrageenan/sterile 0.9% saline is administered and the volume of the injected foot is measured with a displacement plethysmometer connected to a pressure transducer with a digital indicator. Three hours after the injection of the carrageenan, the volume of the foot is again measured. The average foot swelling in a group of drug-treated animals is compared with that of a group of placebo-treated animals and the percentage inhibition of edema is determined (Otterhess and Bliven, Laboratory Models for Testing NSAIDs, in Non-steroidal Anti-Inflammatory Drugs, J. Lombardino, ed. 1985).

EXAMPLE 58

Evaluation of COX-1 and COX-2 Activity In Vitro

The COX-2 inhibition activity of the compounds of this invention can be determined using the following methods.

A. Preparation of Recombinant COX Baculoviruses

Recombinant COX-1 and COX-2 were prepared as described by Gierse et al, [J. Blochem., 305, 479-84 (1995)]. A 2.0 kb fragment containing the coding region of either human or murine COX-1 or human or murine COX-2 is cloned into a BamH1 site of the baculovirus transfer vector pVL1393 (Invitrogen) to generate the baculovirus transfer vectors for COX-1 and COX-2 in a manner similar to the method of D. R. O'Reilly et al (Baculovirus Expression Vectors: A Laboratory Manual (1992)). Recombinant baculoviruses are isolated by transfecting 4 μg of baculovirus transfer vector DNA into SF9 insect cells (2 X 10^8) along with 200 ng of

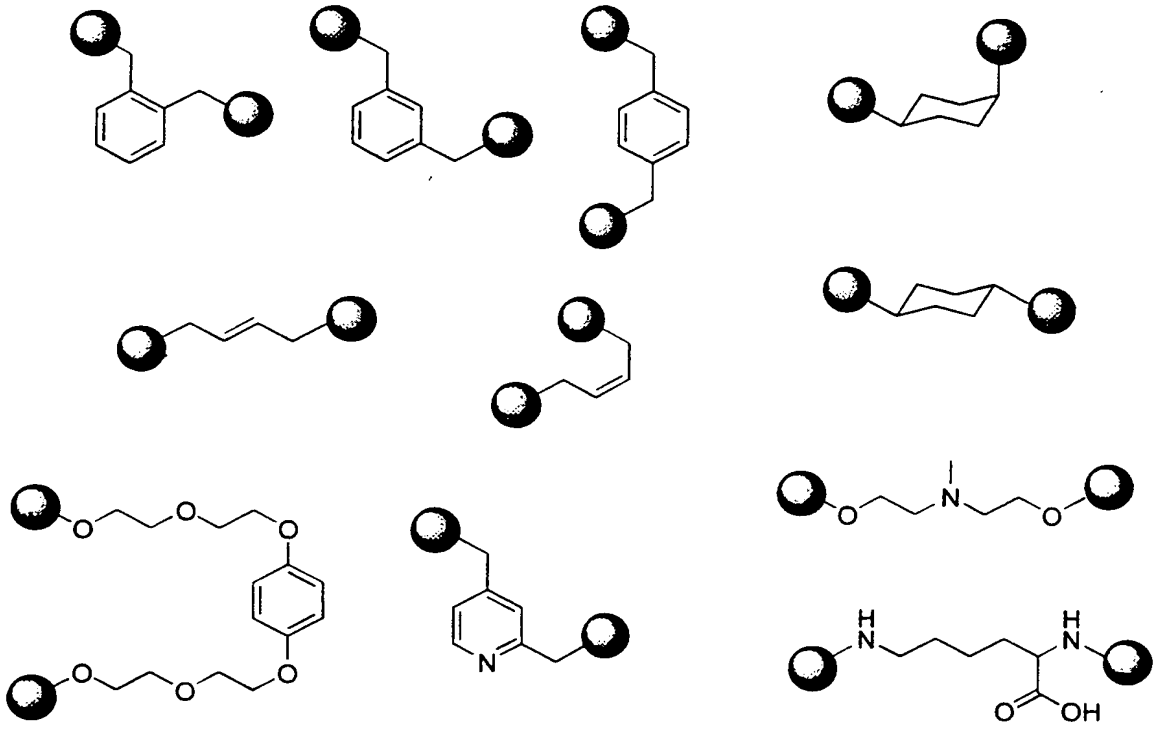
linearized baculovirus plasmid DNA by the calcium phosphate method. See M. D. Summers and G. E. Smith, A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agric. Exp. Station Bull. 1555 (1987). Recombinant viruses are purified by three rounds of plaque purification and high titer (10^7 -10^8 pfu/mL) stocks of virus are prepared. For large scale production, SF9 insect cells are infected in 10 liter fermentors (0.5 X 10^6 /mL) with the recombinant baculovirus stock such that the multiplicity of infection is 0.1. After 72 hours, the cells are centrifuged and the cell pellet homogenized in Tris/Sucrose (50 mM: 25%, pH 8.0) containing 1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS). The homogenate is centrifuged at 10,000 xG for 30 minutes, and the resultant supernatant is stored at -80□C before being assayed for COX activity.

B. Assay for COX-1 and COX-2 Activity

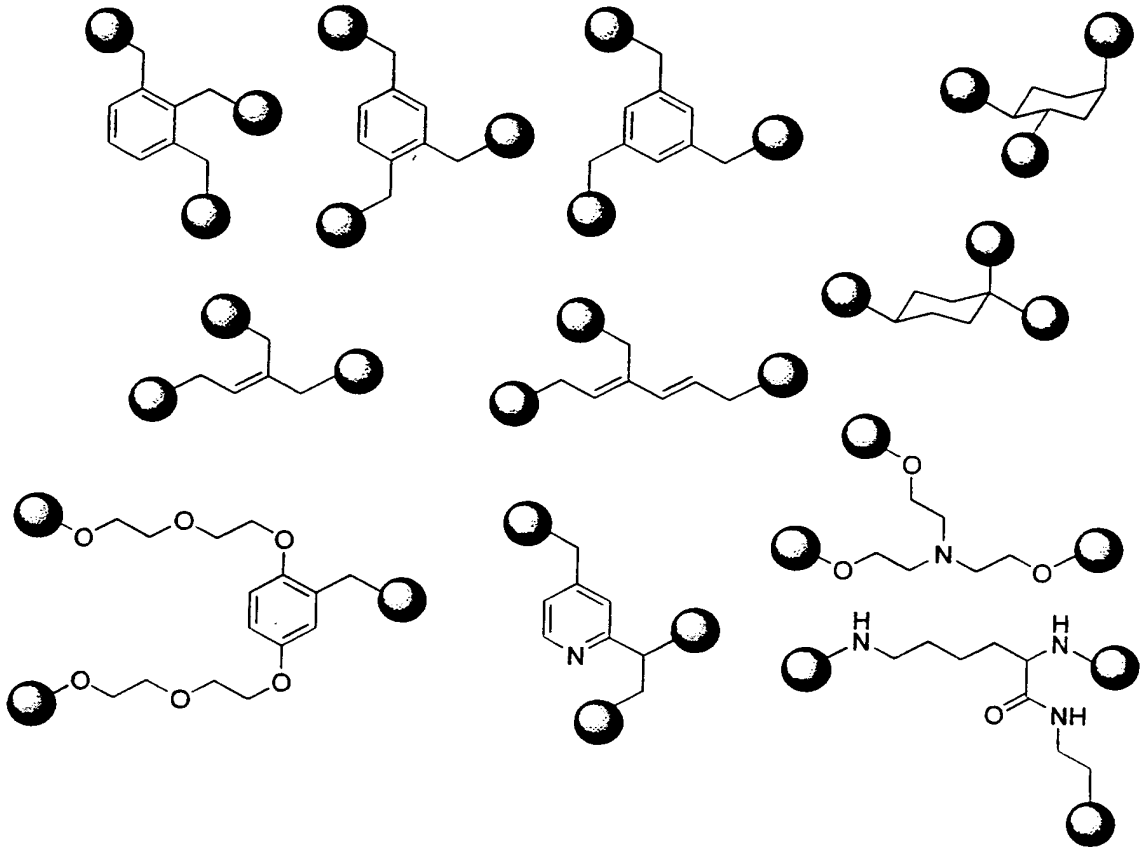
COX activity is assayed as PGE_2 formed/μg protein/time using an ELISA to detect the prostaglandin released . CHAPS-solubilized insect cell membranes containing the appropriate COX enzyme are incubated in a potassium phosphate buffer (50 mM, pH 8.0) containing epinephrine, phenol, and heme with the addition of arachidonic acid (10 μM). Compounds are pre-incubated with the enzyme for 10-20 minutes prior to the addition of arachidonic acid. Any reaction between the arachidonic acid and the enzyme is stopped after ten minutes at 37□C/room temperature by transferring 40 μL of reaction mix into 160 μL ELISA buffer and 25 μM indomethacin. The PGE_2 formed is measured by standard ELISA technology (Cayman Chemical).

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

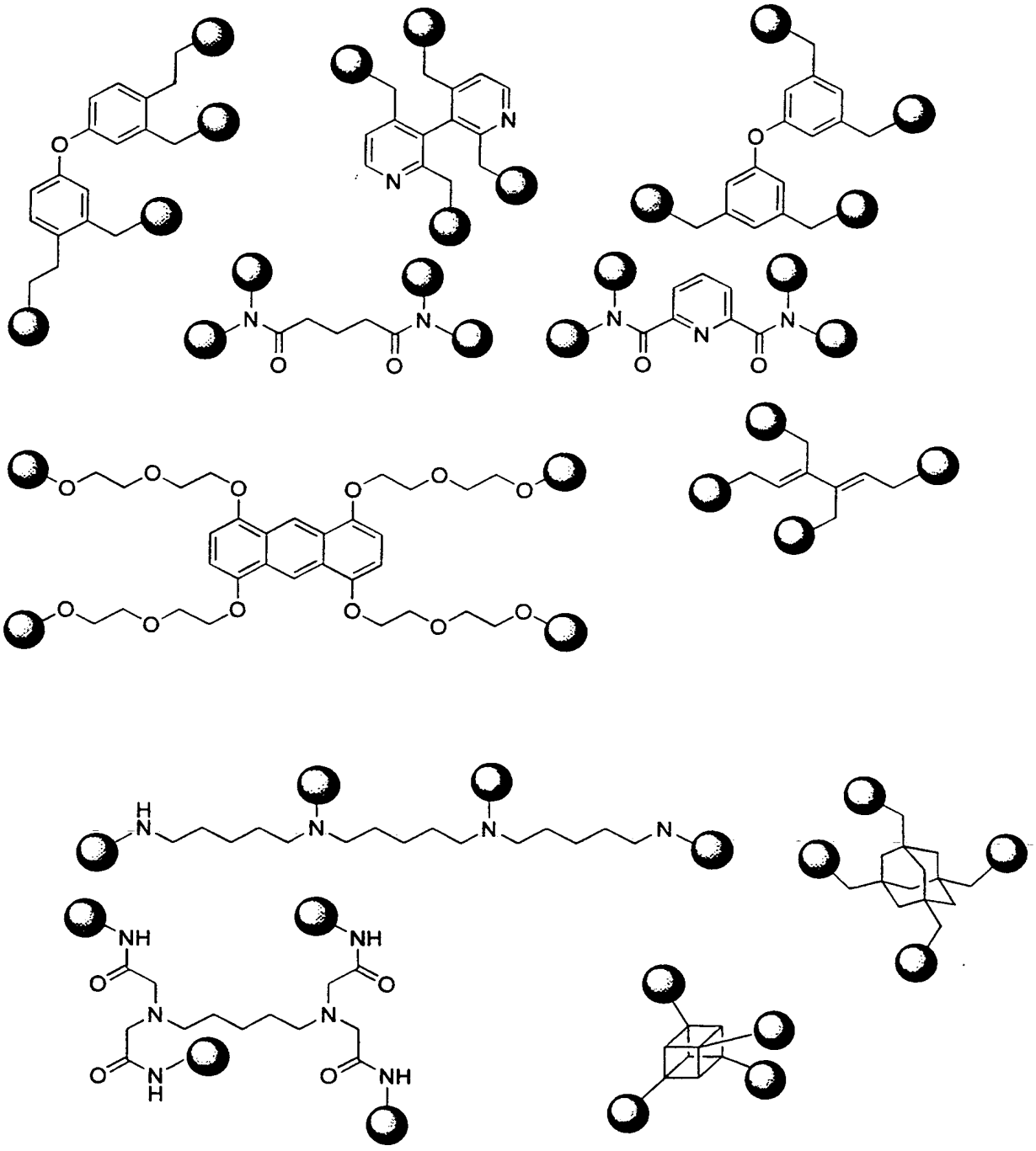
Examples of dimeric display



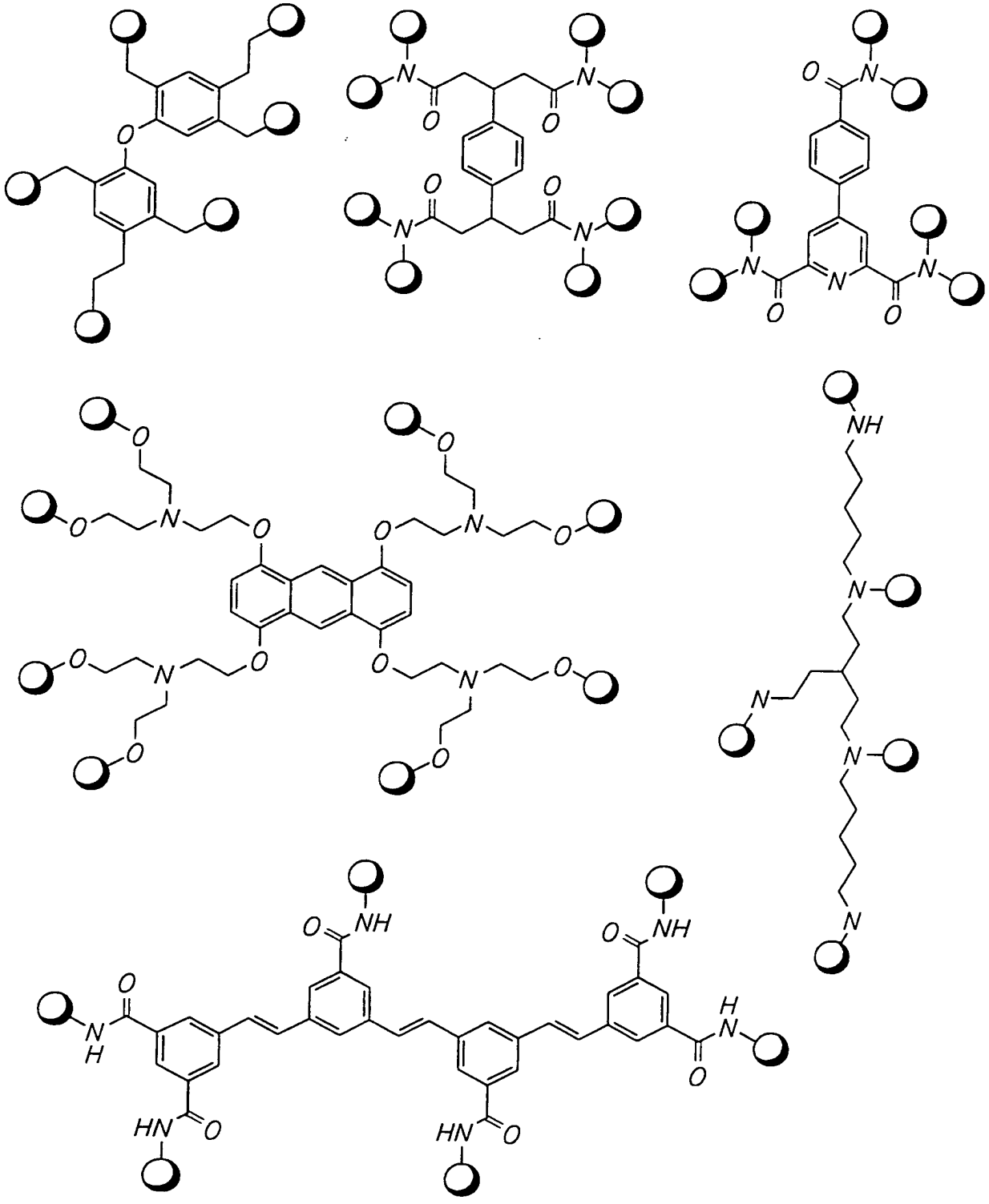
Examples of trimeric display



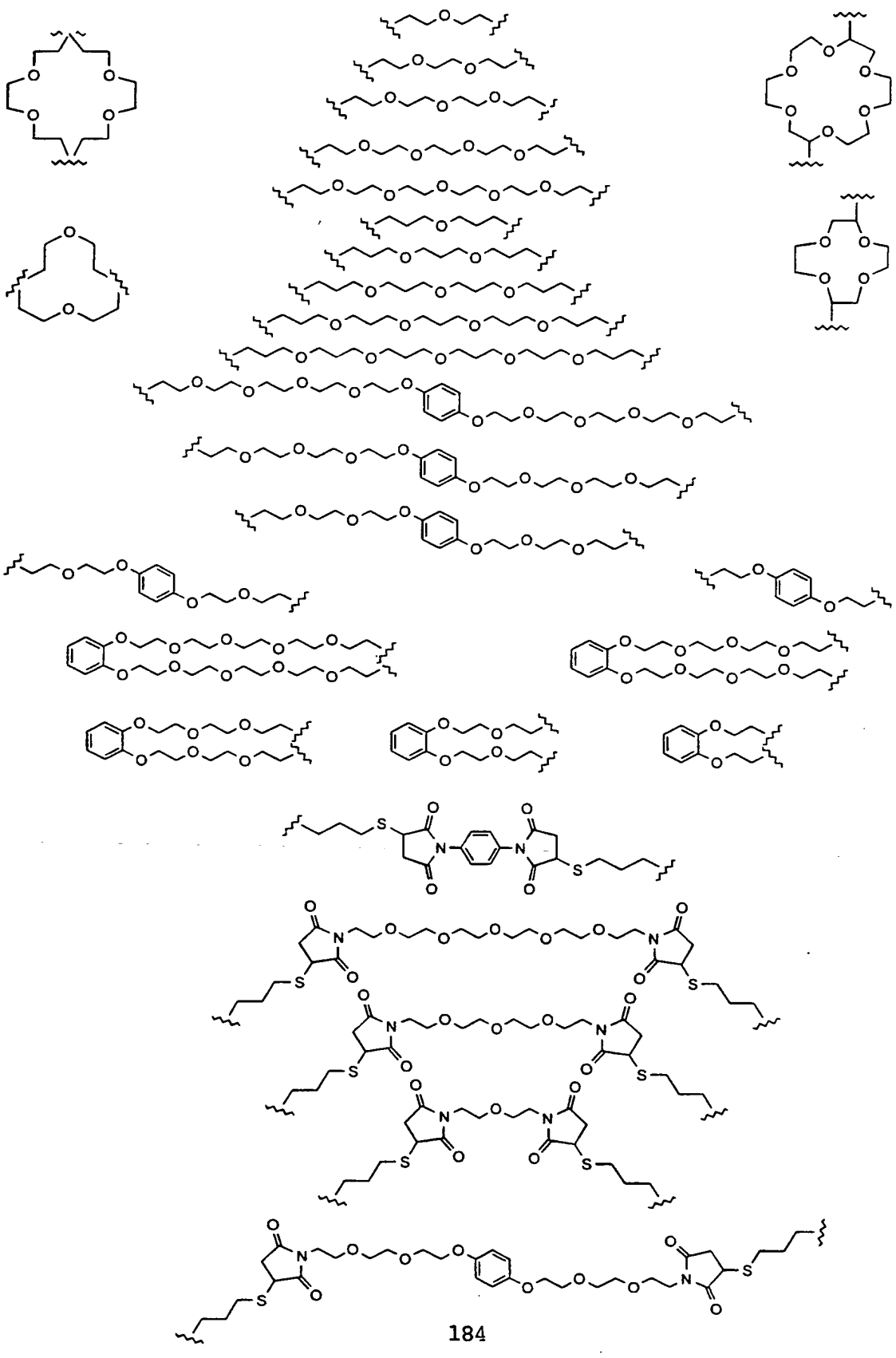
Examples of tetrameric display



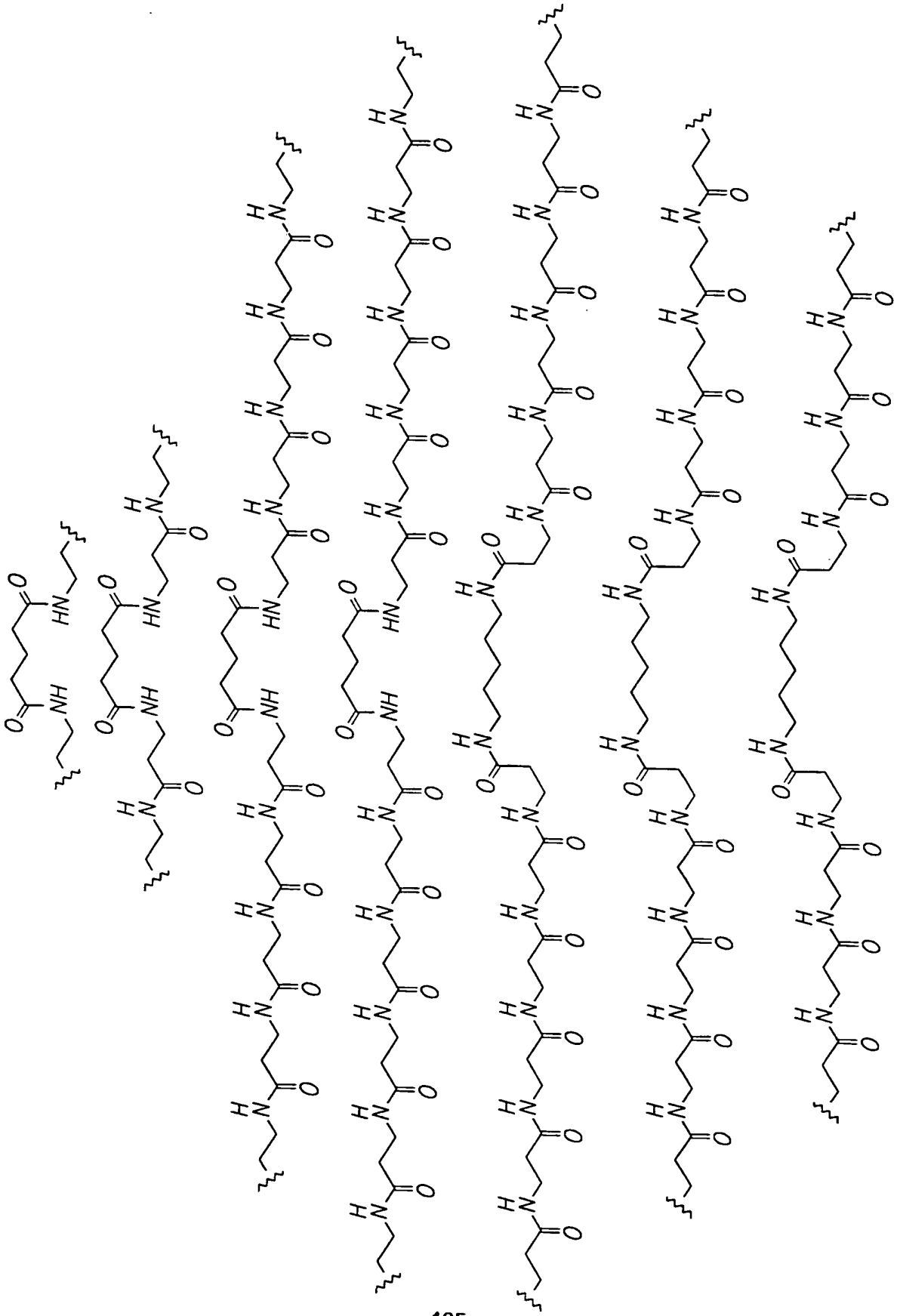
Examples of higher order polyvalent display

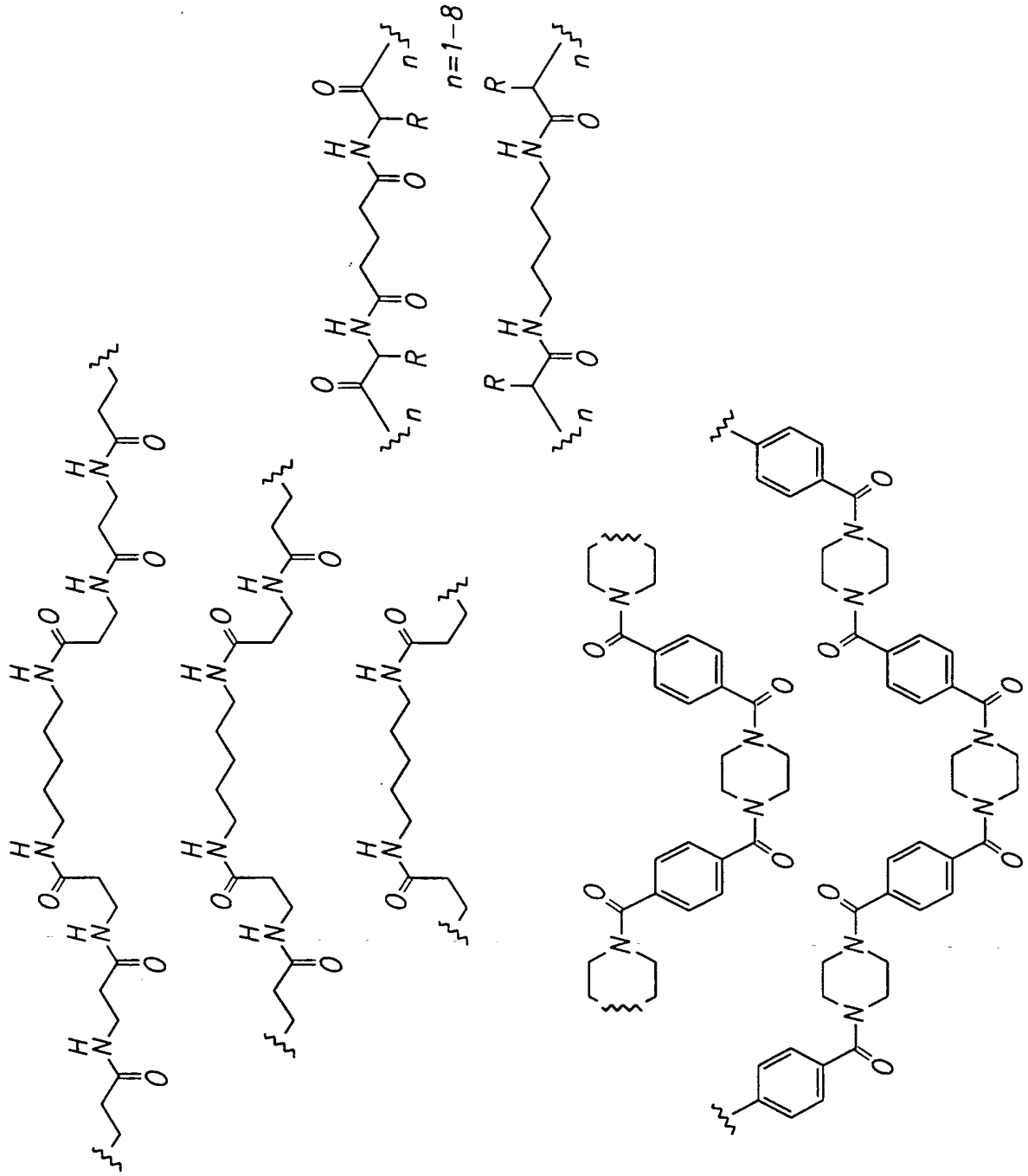


EXAMPLES OF LINKERS



Examples of Linkers





185a

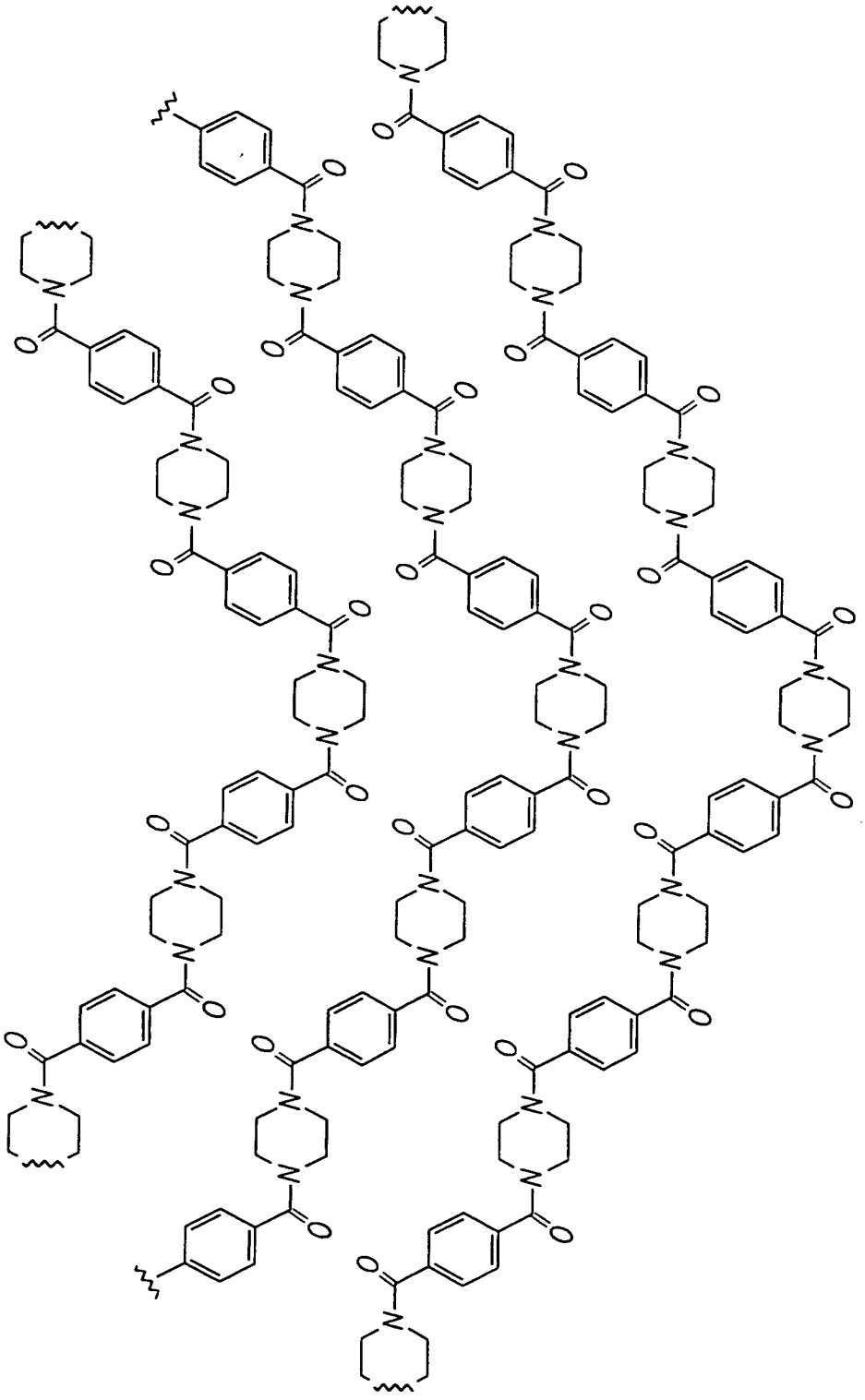


Table 1: Examples of Ligands

Enzyme/ Enzymatic Process	Structural Organization	Therapeutic Indication(s)	Inhibitory Drug(s)
Oxidoreductase Enzymes			
HMG-CoA reductase (1.1.1.34)	Membrane-bound homodimer	Hypercholesteremia	Mevastatin, lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin
3-Beta-hydroxysteroid dehydrogenase (1.1.1.51)		Anti-adrenocorticosteroic	Trilostane
15-hydroxyprostaglandin dehydrogenase (1.1.1.141)	Dimer		
Inosine 5'-phosphate dehydrogenase (1.1.1.205)	Homohexamer/ Homotetramer	Anti-espiratory syncytial virus	Ribavirin (monophosphate form)
Glycerol phosphate oxidase (1.1.3.21)		Anti-protozoal	Suramin
Succinic semialdehyde dehydrogenase (1.2.1.16)	Multimer	Complex partial seizures	Valproic acid
Steroid 5-alpha reductase 2 (1.3.99.5)		Prostatic hypertrophy Male-pattern baldness	Finasteride
Steroid 5-alpha reductase 1 (1.3.99.5)			
Monoamine oxidase (1.4.3.6)	Membrane-bound dimer	Migraine Depression	Phenelzine, isocarboxazid tranylcypromine, selegiline
Dihydrofolate reductase (microbial) (1.5.1.3)		Anti-parasite Anti-bacterial	Chloroguanide, pyrimethamine Trimethoprim
Dihydrofolate reductase (1.5.1.3)	Monomer	Anti-cancer Psoriasis	Methotrexate
Trypanathione reductase (1.6.4.8)		Anti-protozoan	Melarsoprol
Peroxidase (1.11.1.7)		Hyperthyroidism	Propylthiouracil, methimazole, carbimazole
12-Lipoxygenase (1.13.11.31)	Membrane-bound?		
5-Lipoxygenase (1.13.11.34)	Membrane-bound?	Inflammation	Zileuton, docebenone, ICI-D2318, MK-0591, MK-886, piripost, tenidap
Nitric Oxide Synthase (1.14.13.39)	Homodimer		
Cyclooxygenase 1 (1.14.99.1)	Membrane-associated homodimer	Inflammation	NSAIDs, aspirin, ibuprofen, flurbiprofen, indomethacin, acetaminophen, tolmetin, mefanmic acid
Cyclooxygenase 2 (1.14.99.1)	Membrane-associated homodimer (inflamed tissue)	Inflammation	See above
Squalene monooxygenase (1.14.99.7)	Membrane-bound	Anti-fungal	Terbinafine
Cytochrome P450-dependent sterol 14-alpha methyl demethylase (Cytochrome P450 17-alpha or Cytochrome P450 see enzyme) (1.14.99.9 or 1.14.15.6)	Membrane-bound complex with flavoprotein	Anti-fungal Anti-parasitical	Ketoconazole, fluconazole, itraconazole, clotriamzole, miconazole
Cytochrome P450 11-beta		Anti-cancer (hypercorticism in adrenal neoplasm or ectopic ACTH production)	Metyrapone (METPIRONE)
Ribonucleoside diphosphate reductase (1.17.4.1)	Heterodimer	Anti-cancer (myeloproliferative disorders)	Hydroxyurea

Table 2

Exemplary Enzyme Assays

Enzyme Type	Enzyme	Experimental Design	Assay	Reference
Oxidoreductase Enzymes	HMG-CoA reductase	*In vitro*	Rat microsomes isolated from rat liver and treated with HMG-CoA reductase inhibitors Pharmacokinetics of inhibitors of enzyme activity determined	Bischoff, H., et al. 1997 Cerivastatin Pharmacology of a novel synthetic and highly active HMG-CoA reductase inhibitor Atherosclerosis. 135(1):11
	3-β-hydroxysteroid dehydrogenase	*In vitro*	Activity and inhibition of β-hydroxysteroid dehydrogenase in human skin was determined with a radioenzyme assay	Taoth, I., et al. 1997 Activity and inhibition of 3-beta-hydroxysteroid dehydrogenase in human skin. Skin Pharm. 10(3):160
	15-hydroxyprostaglandin dehydrogenas	*In vitro*	Inhibition of 15-hydroxyprostaglandin dehydrogenase was determined by standard kinetic analysis	Nagai, K., et al. 1989. Endogenous inhibitor of 15-hydroxyprostaglandin dehydrogenase in human placenta. Prostaglandins, Leukotrines and Essential Fatty Acids, 38(3):157
	Inosine-5'-phosphate dehydrogenase	*In vitro*	Inosine-5'-phosphate dehydrogenase was studied in leukemic patients treated with an inhibitor of the enzyme	Tricot, GJ, 1989. Biochemically directed therapy of leukemia with tiazofurin, a selective blocker of inosine-5'phosphate dehydrogenase activity. Cancer Research, 49(13):3696.
	Glycerol phosphate oxidase	*In vitro*	Effect of inhibitors of glycerol-3-phosphate oxidase activity was determined by standard methods	Grady, RW, et al., 1986. P-Alkyloxybenzhydroxamic acids, effective inhibitors of the trypanosome glycerol-3-phosphate oxidase. Mol. and Biochem. Parasit. 19(3):231

Table 2

Exemplary Enzyme Assays

Succinic semialdehyde dehydrogenase	*In vitro*	Effect of inhibitors of succinic semialdehyde dehydrogenase, extracted from bovine brain were determined by standard analytical methods	Cho, SW, et al., 1996. Inactivation of an NADPH-dependent succininc semialdehyde reductase by o-phthalaldehde. Febs Letters, 382(1-2):179.
Steroid-5-α-reductase 1 and 2	*In vitro*	The activity of steroid 5-α-reductase 1 and 2, extracted from homogenates of human hypertrophic prostate, was determined by standard analytical methods	Guarna, A., et al. 1997. 19-nor-10-azasteroids: a novel class of inhibitors for human steroid 5-α-reductases 1 and 2. J. Med. Chem. 40(7):1112.
Monoamine oxidase	*In vitro*	The activity of monoamine oxidase was be determined by measuring the the rate of conversion of ^{14}C-indoleacetic acid by liquid scintillation spectrophotometry	Wurtman, RJ, et al., 1963. A sensitive and specific assay for the estimation of monomine oxidase. Biochem. Pharmacol. 12:1439
Dihydrofolate reductase	*In vitro*	Synthetic analoques of trimethoprim were tested for inhibitory effects on protozoan and mammalian dihydrofolate reductase using standard analytical methods	Rosowsky, A. et al., 1998. 2,4-diamino-6,7-dihydro-5H-cyclopent a[d]pyridmidine analogues of trimethoprim as inhibitors of Pneumocystis carinii and Toxoplasma gondii dihydrofolate reductase. J. of Med. Chem. 41(6):913
6-Phosphogluconate dehydrogenase	*In vitro*	Inhibitors of enzyme activity tested in *T. brucei* and lamb liver extracts	Hanau, S., 1996 6-Phosphogluconate dehydrogenase from Trypanosoma brucei. Kinetic analysis and inhibition by trypanocidal drugs Eur. J. of Biochem, 240(3):592
Peroxidase	*In vitro*	The mode of action of inhibitors of thyroid peroxidase was studied using standard analytical methods	Doerge, DR, 1993. Chemical and enzymatic oxidation of benzimidaoline-2-thiones a dichotomy in the mechanism of peroxidase inhibition Biochem. 32(1):58

Table 2

Exemplary Enzyme Assays

12-Lipoxygenase	*In vitro*	Inhibition of 12-lipoxygenase, from feline pulmonary bed, decreased experimentally induced vasoconstriction	Kaye, AD, 1997. Effects of phospholipase A-2, 12-lipoxygenase, and cyclooxygenase inhibitors in the feline pulmonary bed. Amer. J. Physiol, 272(4 part 1):1573
5-Lipoxygenase	*In vitro*	The activity of 5-lipoxygenase was determined with an *in vitro* glucurolosyltransferase assay	Bouska, JJ, et al, 1997 Drug Met And Disposition 25(9):1032
Nitric oxide synthase	*In vitro*	Effect of inhibitors of nitric oxide synthase on seizures studied in rats	Alexander, CB, 1998. Further studies on anti- and proconvulsant effects of inhibitors of nitric oxide synthase in rodants. Eur. J. Pharm. 344(1):15 4 part 1) 1573

Table 2

Exemplary Enzyme Assays

Cyclooxygenase	*In vitro*	Inhibitors of inflammation, induced in dogs, were tested. The effect of the agents on the synthesis of inflammatory cytokines determined	Kirchner, T., et al, 1997. Evaluation of the antiinflammatory activity of a dual cylcooxygenase-2/5-lipooxygenase inhibitor, RWJ63556, in a canine model of inflammation. J. Pharm. Exp. Ther. 282(2):1094
Squalene monooxygenase	*In vitro*	Inhibitors of the activity of squalene synthase induced increases in mRNA for several hepatic enzymes, including squalene synthase	Ness, GC, et al, 1994. Effect of squalene synthase inhibition on the expression of hepaticcholesterol biosynthetic enzymes, LDL receptor, cholesterol and 7 alpha hydroxylase. Arch. Biochem. Biophys. 311(2):277
Cytochrome P450	*In vitro*	Activity of protease inhibitors on cytochrome P450 evaluated *in vitro*	von Moltke, LL, et al. 1998. Protease inhibitors of human cytochrome P450 high risk associated with ritonavir. J. Clin. Pharm. 38(2):106
Cytochrome P450 11-B	*In vitro*	Metyrapone, an inhibitor of the cytochrome P-450 11 beta hydroxylase system, was studied in preparation of bovine adrenal cortical mitochondria	Hays, SJ, et al, 1984. Structure-activityrelationship study of the inhibition of adrenalcortical 11 beta-hydroxylase by new metyrapone derivatives, J. Med. Chem, 27(1):15
Ribonucleotide diphosphate reductase	*In vitro*	The inhibition of ribonucleotide diphosphate reductase was evaluated in a new assay system	Jong, AY, et al, 1998. A simple and sensitive reibonucleotide reductase assay. J. Biomed. Sci. 5(1):62
Aromatase	*In vitro*	Effects of aromatase inhibitor studied in cultured human adrenal cells	Lamberts, SW, et al, 1989. The new aromatase inhibitor, CGS-16949A, suppresses aldosterone and cortisol production by human adrenal cells in vitro. J. Clin. Metab, 69(4):696

Table 2

Exemplary Enzyme Assays

Tranferases	Catechol-O-methyl transferase	*In vitro*	Pharmacokinetics and inhibitory effect of entacapone on catechol-O-methyltransferase was studied in red blood cells	Keranen, T. et al, 1994. Inhibition of soluble catechol-O-methyltransferase and single dose pharmacokinetics after oral and intravenous administration of entacapone. Eur. J. Clin. 46(2):151
	Glycinamide ribonucleotide transformylase	*In vitro*	Effect of a variety of enzyme inhibitors tested *in vitro*	Boger, DL, et al, 1997. 10-Formyl-5,8,10-trideazafolic acid (10-formyl-TDAF): a potent inhibitor of glycinamide ribonucleotide tranfromylase. Bioorg. And Med. Chem. 5(9):1817
	Ribosomal protein biosynthesis (50 S subunit)	*In vitro*	Mechanism of inhibition of enzyme evaluated *in vitro*	Drainas, D, et al, 1987. Inhibition of reibosomal peptidyltransferase by chloramphenicol. Kinetic studies. Eur. J. Biochem. 164:(1)53
	Ribosomal protein biosynthesis (aminoacyl tRNA on 30 S ribosomal unit)	*In vitro*	Mechanism of inhibitory effect of macrolides on protein synthesis evaluated	Mennings, JR, 1985. Functional consequences of binding macrolides to ribosomes. J. Antimicrob. Chemother. 16(Suppl A):23
	Ribosomal protein biosynthesis (30 S subunit	*In vitro*	Protein synthesis in *H. influenze* was inhibited by tobramycin	Levy, J, et al, 1986. Effect of tobramycin on protein synthesis in 2-deoxy-streptamine aminoglycoside-resistant clinical isolates of *Haemophilus influenzae*. Antimicro. Agents and Chemo. 29(3):474
	Ribosomal protein biosynthesis (soluble protein factors)			

Table 2

Exemplary Enzyme Assays

Hypoxanthine-guanine phosphoribosyltransferase	*In vitro*	Effect of inhibitors of hypoxanthine-quanine phosphoribosyltransferase, on the growth, of parasitic protozoans was studied *in vitro*	Somoza, JR, et al, 1998. Rational design of novel antimicrobials: blocking purine salvage in a parasitic protozoan. Biochem. 37(16):5344
Dihydropteroate synthase	*In vitro*	A variety of inhibitors of dihydrofolate reductase were studied in dihydrofolate from *T. gondii*	Chio, LC, et al, 1993. Identification of highly potent and selective inhibitors of *Toxoplasma gondii* dihydrofolate reductase. Antimicrob. Agents and Chemotherapy, 37(9):1914
Leukotriene C-4 synthase	*In vitro*	The effect of enzyme inhibitors studied in rat basophilic leukemia cells	Hamasaki, Y, et al, 1996. Inhibitors of leukotriene synthesis by azelastine. Annals of Allergy, Asthma, and Immunology, 76(5):469
GABA transaminase	*In vitro*	Hydroxyamino analogues of gamma-aminobutyric acid were synthesized and tested for inhibition of the transaminase	Fushiya, S, et al, 1997. A novel class of potent gamma-aminobutyric acid aminotransferase inhibitor, 3-(hydroxyamino)propylamine and analogues Bioorg and Med Chem, 5(11):2089

Table 2

Exemplary Enzyme Assays

	Guanylyl kinase	*In vitro*	Effect of inhibitors of protein typrosine kinases determined	Wolbring, G, et al, 1994. Inhibition of GTP-utilizing enzymes by tyrhostins J. Biol. Chem., 269(36):22470
	Beta subunit of DNA-dependent RNA polymerase	*In vitro*		Adhya, S, 1996 RNA polymerase and associated factors. Methods Enzymol., 23(Part A):377
	Reverse transcriptase	*In vitro*	Nuceloside analogues and nonnucleoside reverse transcriptase inhibitors were evaluated for effect on several immunodeficiency viruses	Isaka, Y, et al, 1998. Construction of the chimeric reverse transcriptase of simian immunodeficiency virus sensitive to nonnucleoside reverse transcriptase inhibitor. Microbiol. andImmunol, 42(3):195
	D,D-transpeptidases (PBP 1-6)	*In vitro*	Tigemonam and temocillin, molecules that bind to PBPs and block cell wall synthesis by bacteria, were studied	Bush, K, et al, 1987. Improved sensitivity in assays for binding of novel beta-lactam antibiotics to penicillin-binding proteins of *Escher ichia coli*. Antimicrob. Agents And Chemother. 31:1271
	Transglycosylase	*In vitro*	The inhibition of cell wass synthesis was studied using wall-membrane particulate fractions	Brotz, H, 1997. The lantibiotic mersacidin inhibitspeptidoglycan biosynthesis at the level of transglycosylation Eur. J. Biochem. 246:193
Hydrolases	Phospholipase A2	*In vitro*	Inhibition of phospholipase A-2, from feline pulmonary bed, decreased experimentally induced vasoconstriction	Kaye, AD, 1997. Effects of phospholipase A-2, 12-lipoxygenase, and cyclooxygenase in-hibitors in the feline pulmonary bed Amer J. Physiol, 272(4 part 1):1573

Table 2

Exemplary Enzyme Assays

Acetylcholinesterase	*In vitro*	Inhibition of acetylcholinesterase measured by standard analytical methods	Ellman, G, let Al., 1961. A new and rapid colormetric determination of acetylcholinesterase activity. Biochem. Pharm. 7:88
Cholinesterase	*In vitro*	Enzyme inhibition by a variety of drugs determined	Chemnitius, JM, 1997. Mipafox differential inhibition assay for heart muscle cholinesterases: substrate specificity and inhibition of three isoenzymes by physostigmine and quinidine. Gen. Pharmac. 28(4):567
Phospholipase C	*In vivo*	The effect of inhibition of phospholipase C was studied *in vivo* in mice in endotoxin-induced shock	Tschaikowsky, K, et al, 1998. Modulation of mouse endotoxin shock by inhibition of phosphatidylcholine-specific phospholipase C. J. of Pharm. And Exp. Ther., 285(2):800
Type III cyclic AMP phosphodiesterase	*In vivo*	The inhibitory effect of amrinone on cyclic AMP phosphodiesterase activity was tested *in vivo*. Amrinone retarded death from viral myocarditis in experimentally infected mice	Seta, Y, et al, 1997. Effect of amrinone on murine viral myocarditis. Res. Commun. Mol. Pathol. Pharmacol. 95(1):57-66
Type V cyclic nucleotide phosphodiesterase	*in vitro*	Mechanism of action of inhibitors evaluated	Corbin, JD, et al, 1998. A p;hotoaffinity probe covalently modifies the catalytic site of the cGMP-binding of cGMP-specific phosphodiesterase PDE 5. Cell Biochem. Biophys., 29(1-2):145
Type I cyclic nucleotide phospodiesterase	*In vitro*	Effect of isoenzyme-selective inhibitors on cyclic nucleotide levels determined in human smooth muscle slices	Truss, MC, et al, 1996. Cyclic nucleotide phosphodiesterase isoenzymes in the human detrusor smooth muscle. II. Effect of various PDE inhibitors on smooth muscle tone and cyclic nucleotide levels *in vitro*. Urol. Res. 24(3):129

Table 2

Exemplary Enzyme Assays

Type IV cyclic AMP phosphodiesterase	*In vitro*	The responses of ventricle strips from guinea-pig heart were studied in the presence of inhibitor of pphosphodiesterase	Collado, MC, et al, 1998. Functional and biochemical evidence for diazepam as a cyclic nucleotide phosphodiesterase type 4 inhibitor. Brit. J. of Pharm. 123(6):1047
Glucoamylase	*In vivo*	A novel compound, that inhibits alph-glucohydrolases, was tested *in vivo*	Rhinehart, BL, et al, 1987. Inhibition of intestinal disaccharidases and suppression of blood glucose by a new alpha-glucolydrolase inhibitor--MDL25, 637. J. Pharm. And Exp. Ther., 24(3):915
Neuraminidase	*In vitro*	Synthetic inhibitors of neuraminidase were studied by X-ray crystallography	Chand, P, et al, 1997. Design and synthesis of benzoic acid derivatives as influenza neuramindase inhibitors using structure based drug design J. Med. Chem., 40(25):4030

Table 2

Exemplary Enzyme Assays

α-glucosidase	*In vitro*	Effect of inhibitor determined using enzymes from rabbit heart extracts	Arai, M, met al, 1998. N-methyl-1-deoxynojirimycin (MOR 14), an alpha glucosidase inhibitor, markedly reduced infarct size in rabbit hearts Circulation, 97(13):1290
Leukotriene A hydrolase	*In vitro*	HPLC was used to measure production of leukotrienes in a cell line incubated with enzyme inhibitors and and	Hamasaki, Y, et al, 1996. Inhibition of leukotriene syntheis by azelastine Annals of Allergy, Asthma, and Immunol., 76(5):469
Angiotensin converting enzyme	*In vivo*	The effects of an ACE inhibitor studied *in vivo*	Honda, Y, et al, 1997. Alacepril, an angiotensin-converting enzyme inhibitor, prevents cerebral vasospasm in subarachnoid hemorrhage model in rats. Methods and Findings in Exp. And Clin. Pharm., 19(10):699
dihydropterate synthase	*in vitro*	Inhibition of dihydropteroate synthase by sulfanilanilides with 3',5'-halogen substitutions evaluated	Chio, LC, et al, 1996. Identification of a class of sulfonamides highly active against dihydropteroate synthase from Toxoplasmosis gondii, Pneumocytis carinii, and Mycobaterium aviu. Antimicrob. Agents and Chemother. 40(3):727
D,D-carboxypeptidase (PBP 3-7)	*in vitro*	Inhibition of enzyme, expressed by *Ochrobactrum anthropi* studied	Kato, AY, et al, 1992. Structural similarity of D-aminopeptidase to caroxypeptidase DD and beta-lactamases. Biochem. 11(8):2316

Table 2

Exemplary Enzyme Assays

Coagulation proteases Factor 1xa Factor VIIa Factor Xa Thrombin	*In vitro*	Inhibitory effect of a variety of enzyme inhibitors studied *in vitro*	Kam, CM, et al, 1994. Mechanism-based isocoumarin inhibitors for blood serine prteases. Effect of the 7-substituent in 7-amino-4-chloro-3-(isothiureidoalkosy)isocoumarins on inhibitory and anticoagulant potency. J. Med. Chem., 37(9):1298
Hemoglobin protease (plasmepsin I and II)	*In vitro*	The kinetics of the activation and inhibition of enzyme activity studied *in vitro*	Moon, RP, et al, 1997. Expression and characterisation of plamepsin I from Plasmodium falciparum. Eur. J. of Biochem., 244(2):552
	In vitro	Structural and functional studies performed on inhibitors of plasmepsin I and II *in vitro*	Silva, AM, et al, 1996. Structure and inhibition of plasmepsin II, a hemoglogin-degrading enzyme from Plasmodium falcparum. PNAS., 93(19):10034
Neutral endopeptidase	*In vivo*	CGS 30440, an inhibitor of angiotensin-converting enzyme, demonstrated antihypertensive activity in spontaneously hypertensive rats	Webb, RL, et al, 1997. Effects of the novel dual inhibitor of neutral endopeptidase and angiotensin-converting enzyme, CGS 30440 on blood pressure and cardiac hypertrophy in spontaneously hypertensive rats. J. Cardiovasc. Pharacol., 30(5):632
β-lactamase	*in vitro*	Kinetics of the inhibitory effect of B-lactamase inhibitors determined with tiem-lill and activity assays	Hoellman, DB, et al, 1998. Activities andtime-lill studies of selected penicillins, beta-lactamase inhibitor combinations, and glycopeptides against Enterococcus facalis Antimicrob Agents and Chemother, 42(2):857

Table 2

Exemplary Enzyme Assays

	Adenosine deaminase	*In vitro*	Effects of structural variation, of inhibitors of adenosine deaminase, were evaluated	Shewach, DS, et al, 1992. Inhibition of adenosine deaminase by azapurine ribonucleosies Biochem. Pharm, 44(9):1697
	Undecaprenyldiphosphatase	*n vitro*		Spencer, J, et al, 1978. Inhibition of lipid-linked saccharide synthesis by bacitracin Arch. Biochem Biophys, 190:829
	Iodothyronine-5'-deiodinase type I	*In vitro*	The effect of inhibitors of type I iodothyronine activity was studied in enzyme extracted from rat liver	Visser, TJ, et al, 1992. Setenouracil derivatives are potent inhibitors of the sclenoenzyme type I iodothyronine deiodinase BBRC 189(3):1362
	Iodothyronine-5'-deiodinase type II	*In vitro*	Structural elements of inhibitors of iodothyronine5'-deiodinase were evaluated *in vitro*	Safran, M, et al, 1993. Structural requirements of iodothyronines for the rapid inactivation and internalization of type II iodothyronine 5'-deiodinase in glial cells J. Biol. Chem., 268(19):14224
Lyases	Glutamic acid decarboxylase	*In vitro*	The stimulatory effect of valproate on glutaminase was studied in isolated dog kidney tubules	Martin, G, et al, 1989. Stimulation of glutamine metabolism by the anti-epileptic drug, sodium valproate, in isolated dog kidney tubules Biochem. Pharm., 38(22):3947

Table 2

Exemplary Enzyme Assays

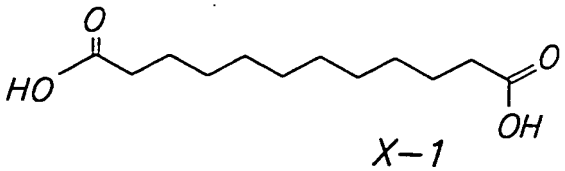
	Carbonic Anhydrase	*In vitro*	The inhibitory effect of sulfenamindo-sulfonamides, on carbonic anhydrase activity, was studied *in vitro*	Supuran, CT, 1997. Sulfenamido-sulfonamides as inhibitors of carbonic anhydrase isozymes I, II and IV J. of Enzyme Inhib., 12(3):175
Isomerases	L-alanyl racemase	*In vitro*	Inactivators of alanine racemase were evaluated in cultures of Gram positive and negative bacteria	Cheung, KS, 1986. Chloroalanyl antibiotic peptides: antagonism of their antimicrobial effects by L-alanine and L-alanyl peptides in gram-negative bacterial J. Med. Chem. 29(10):2060
	Prostacyclin synthase	*In vitro*	The effect of enzyme inhibition on prostacylin formation was evaluated using aortic microsomes	Zou, M, et al, 1997. Tyrosine nitration as a mechanism of selective inativation of prostacyclin synthase by peroxynitrite. Biol. Chem., 378(7):707
	Thromboxane synthase	*In vitro*	Enzyme inhibitors were evaluated *in vitro* using platelets and blood	Brownlie, RP, et al, 1993. ZD1542, a potent thromboxane A2 synthase inhibitor and receptor antagonist *in vitro*. Brit. J. of Pharm., 110(4):1600
	Lanosterol cyclases	*In vitro and In vivo*	*In vitro* and *in vivo* effects of inhibitors of cylcases were tested	Taton, M, et al, 1992. Inhibitors of 2,3-oxidosqualene cyclases. Biochem. 31(34):7872
	DNA gyrases	*In vitro*	Effect of inhibitors, of bacterial gyrases, was studied *in vitro* using time-kill assays 39(12):2822	Nakane, T, 1995. In vitro antibacterial activity of DU-6859a, a new fluoroquinolone. Antimicrob. Agents and Chemo., 39(12):2822
	Topoisomerase II	*In vitro*	In vitro effects of benzene metabolites on human topoisomerase II determined.	Frantz, CE, et al, 1996. Inhibition of human topoisomerase II in vitro by bioactive benzene metabolites. Environ. Health Perspect. 104(6):1319

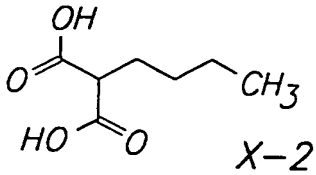
Table 2

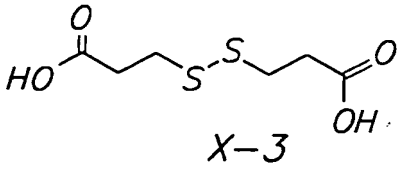
Exemplary Enzyme Assays

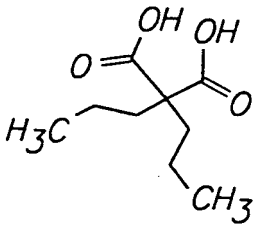
	Fungal isomerase enzymes	*In vitro*	The mode of action of inhibitors of fungal isomerases were studied	Hennig, L, et al, 1998. Selective inactivation of parvulin-like peptidyl-prolyl cis/trans isomerase by juglone. Biochem., 37(17):5953
Ligases	D-alanyl-D-alanine ligase	*In vitro*	Inhibitors of D-alanyl-D-alanine ligase were synthesized and kinetics of effect studied *in vitro*	Chakravarty, PK, 1989 (3-Amino-2-oxoalkyl)phosphonic acids and their analogues as novel inhibitors of D-alanine:D-alanine ligase. J. Med. Chem., 32(8):1886
Unclassified	Plasmodial heme polymerase	*In vivo*	Mice, infected with Plasmodium berghei, were treated with chloroquine and another quinoline derivatives some of which affected the activity of the enzyme	Chou, AC, et al, 1993. Contorl of heme polymerase by cholorquine and other quinolone derivatives. Biochem and Biophys. Res. Commun, 195(1):422

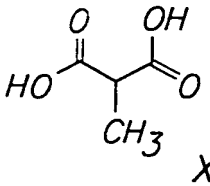
Diacids

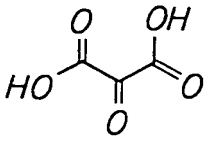

X-1

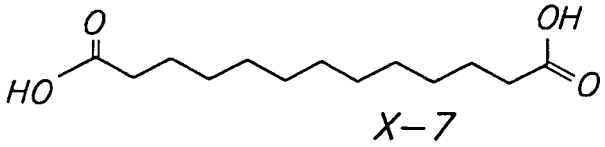

X-2

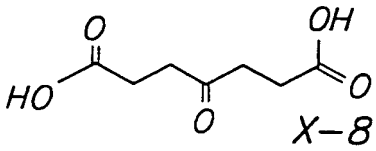

X-3

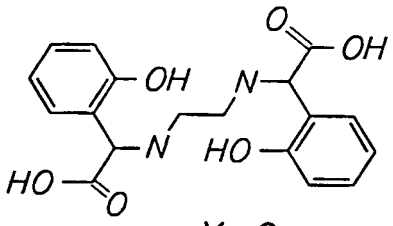

X-4

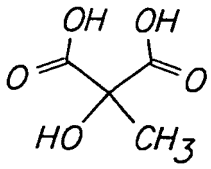

X-5

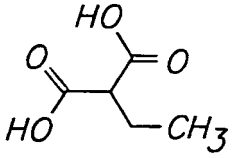

X-6

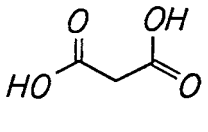

X-7

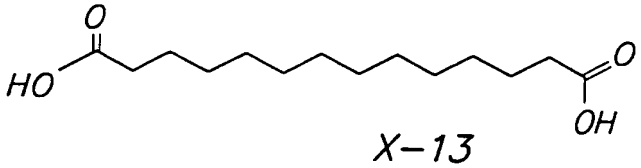

X-8

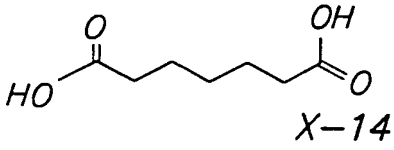

X-9

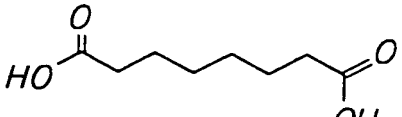

X-10

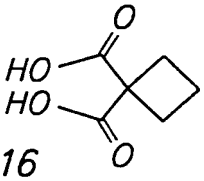

X-11

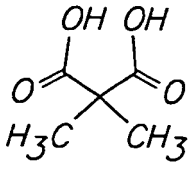

X-12

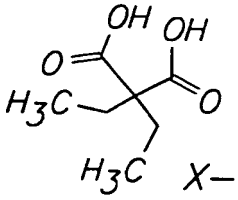

X-13

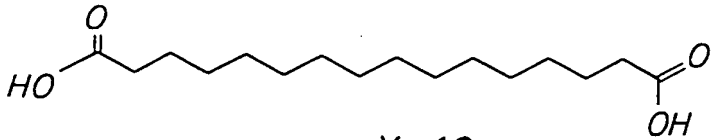

X-14

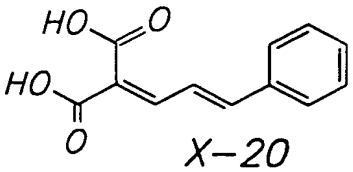

X-15


X-16

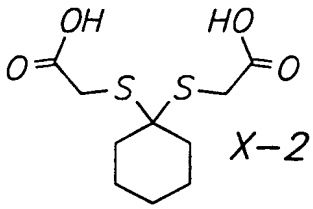

X-17


X-18

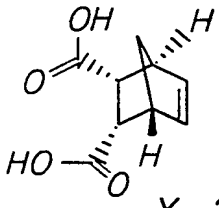

X-19



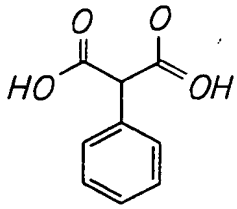
X-20



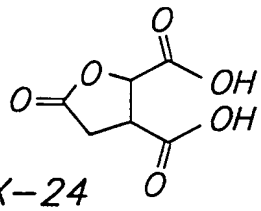
X-21



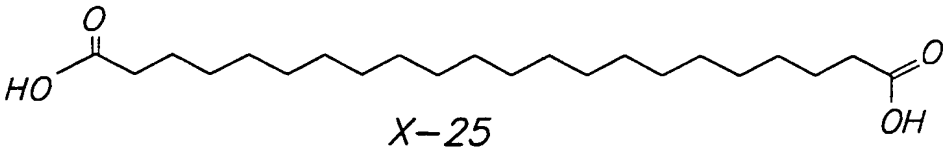
X-22



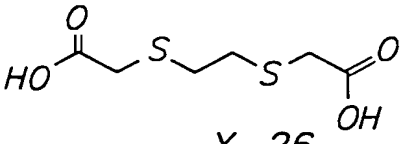
X-23



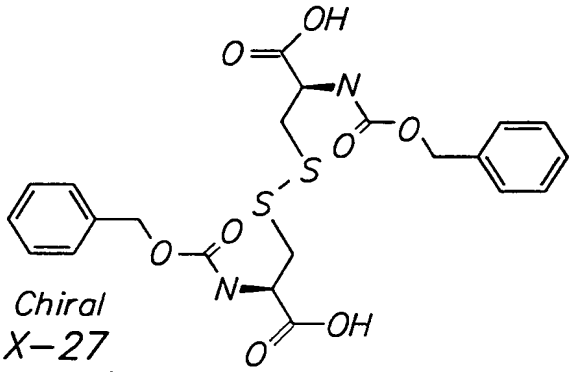
X-24



X-25

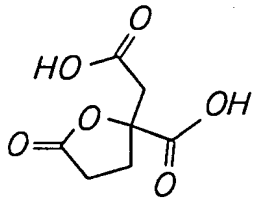


X-26

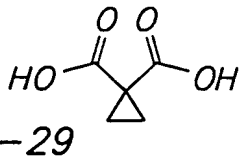


Chiral

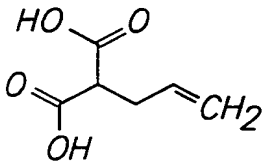
X-27



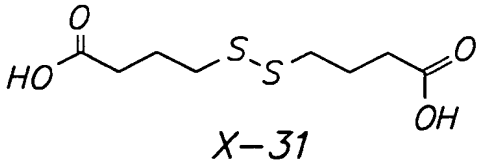
X-28



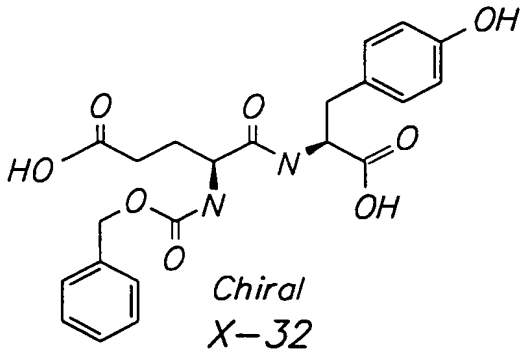
X-29



X-30

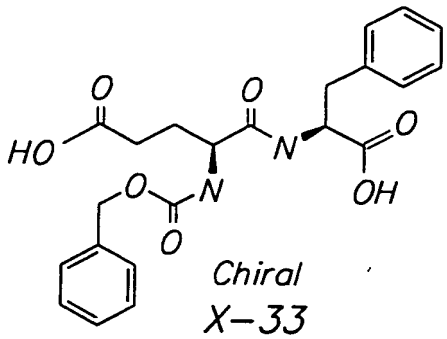


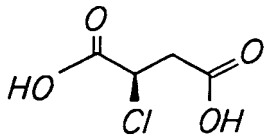
X-31

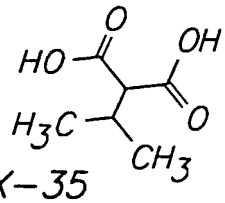


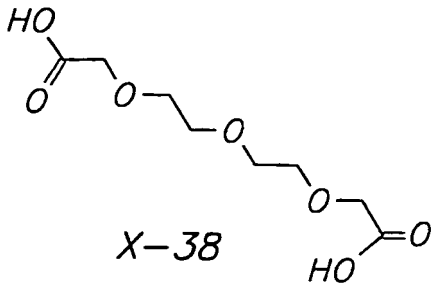
Chiral

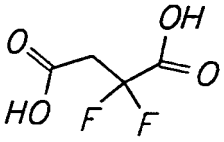
X-32

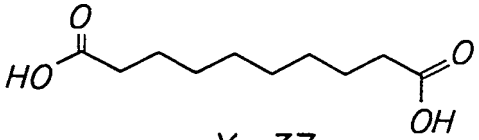

Chiral
X–33

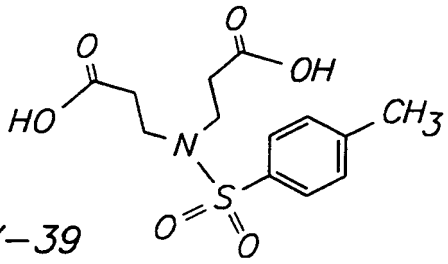

X–34
Chiral

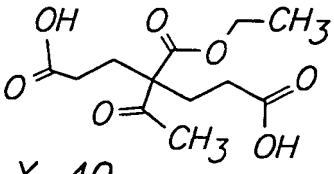

X–35

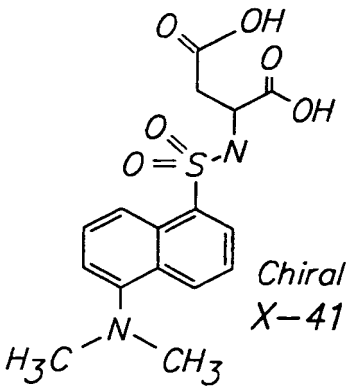

X–38

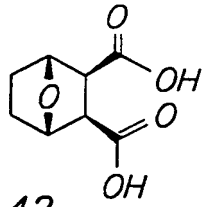

X–36

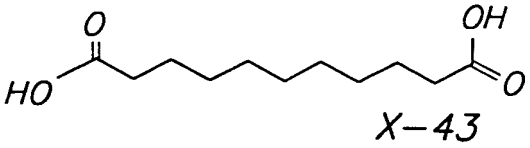

X–37

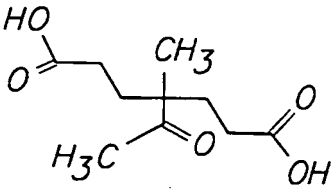

X–39

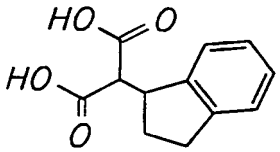

X–40

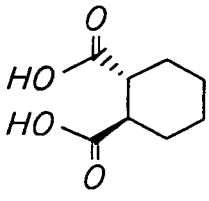

Chiral
X–41

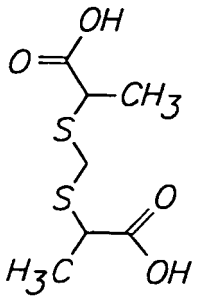

X–42

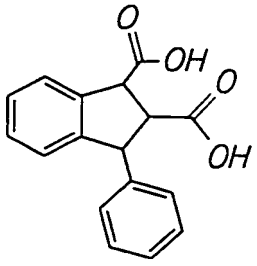

X–43

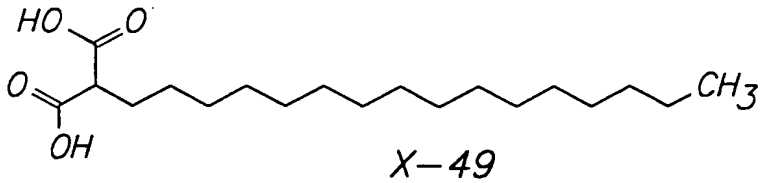

X–44


X–45

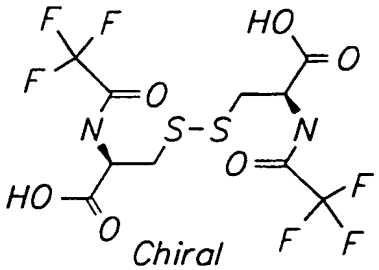

X–46
Chiral


X–47

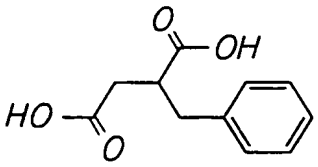

X–48



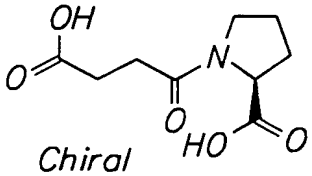
X–49



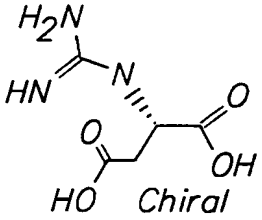
Chiral
X–50



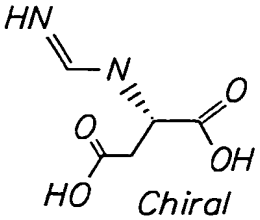
X–51



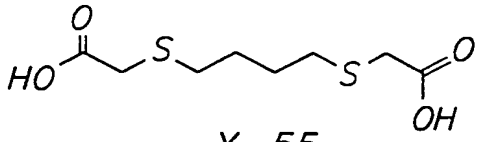
Chiral
X–52



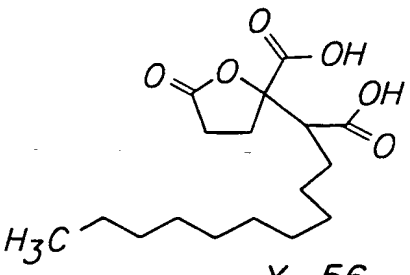
Chiral
X–53



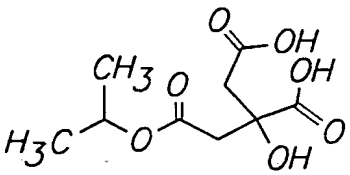
Chiral
X–54



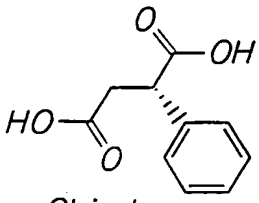
X–55



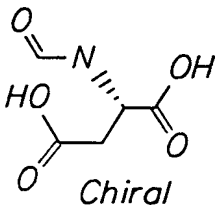
X–56



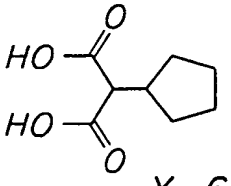
X–57



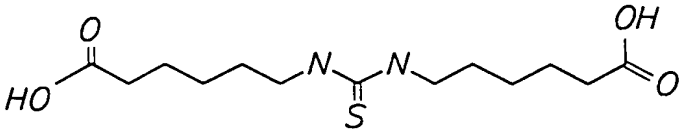
Chiral
X–58



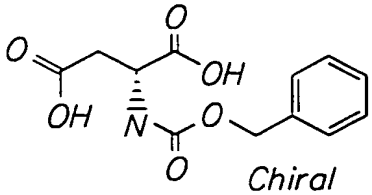
Chiral
X–59



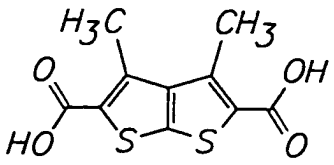
X–60



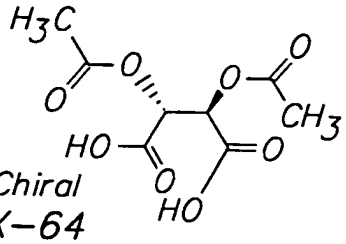
X–61



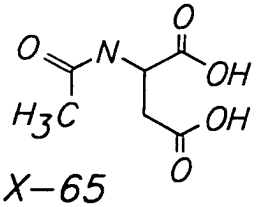
Chiral
X–62



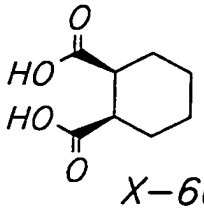
X-63



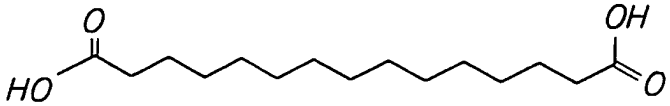
Chiral
X-64



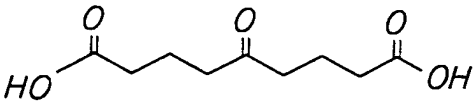
X-65



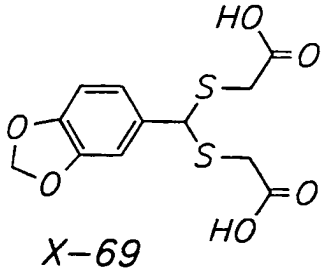
X-66



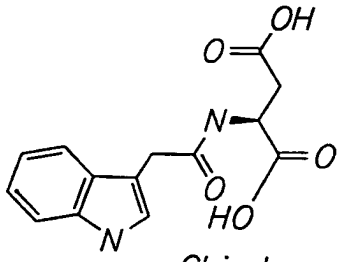
X-67



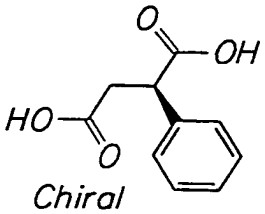
X-68



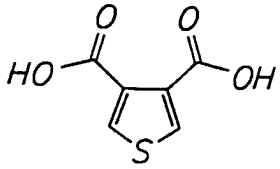
X-69



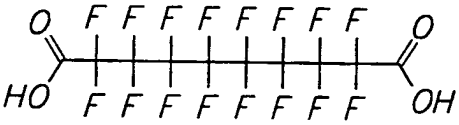
Chiral
X-70



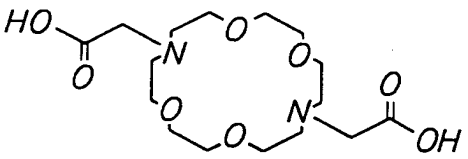
Chiral
X-71



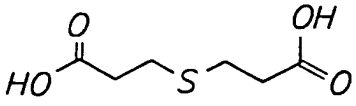
X-72



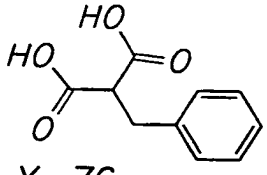
X-73



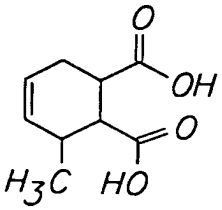
X-74



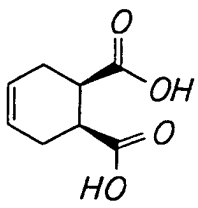
X-75



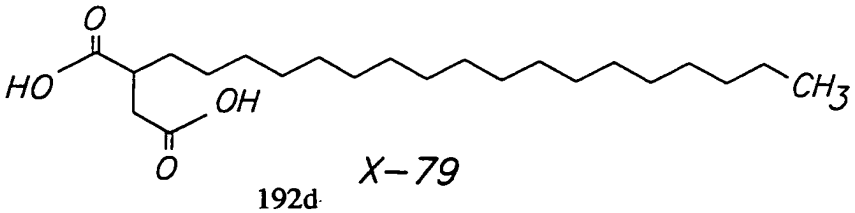
X-76



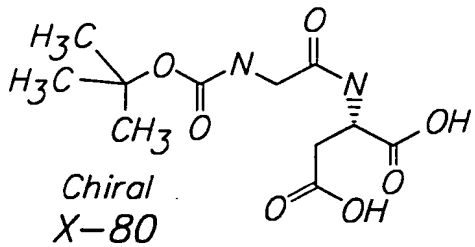
X-77



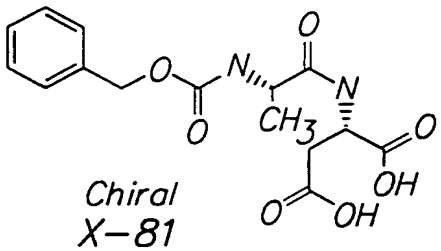
X-78



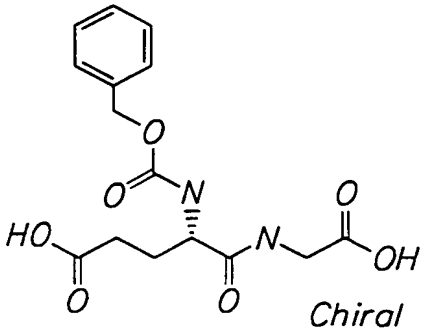
X-79



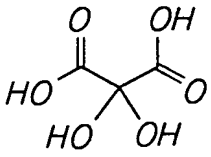
Chiral
X-80



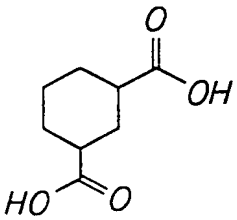
Chiral
X-81



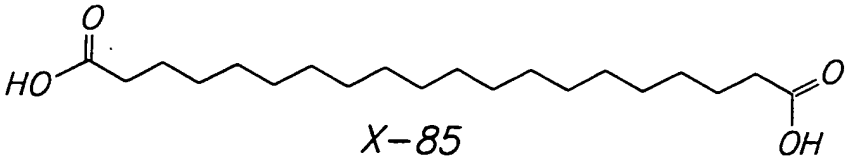
Chiral
X-82



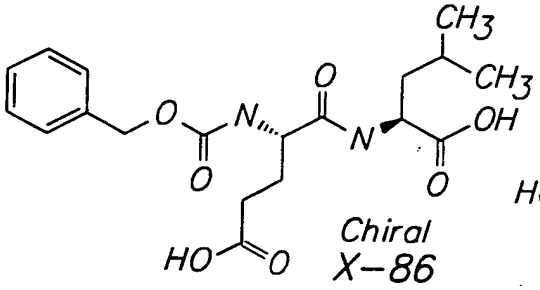
X-83



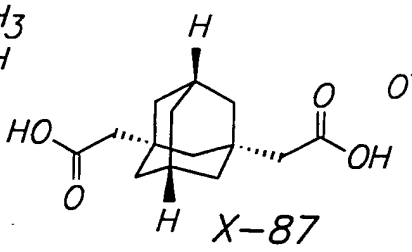
X-84



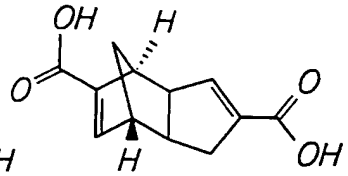
X-85



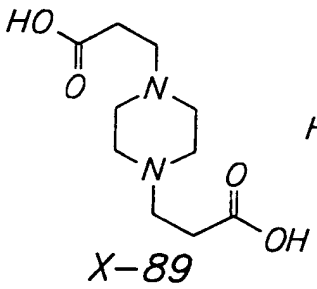
Chiral
X-86



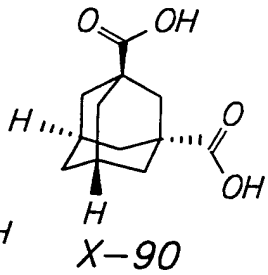
X-87



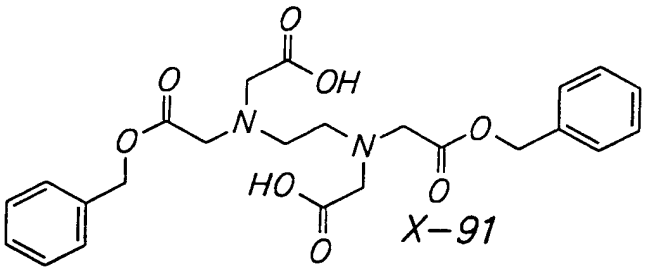
X-88



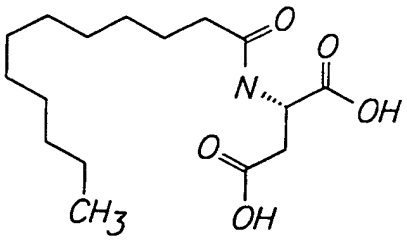
X-89



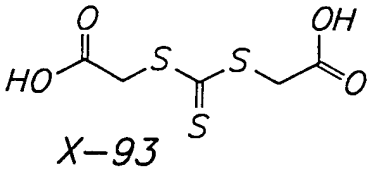
X-90



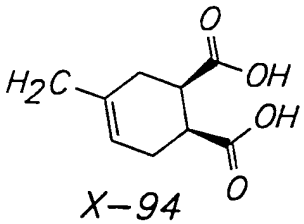
X-91



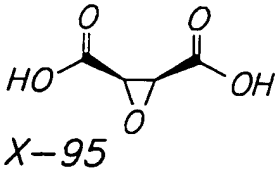
Chiral
X-92



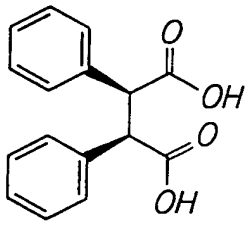
X-93



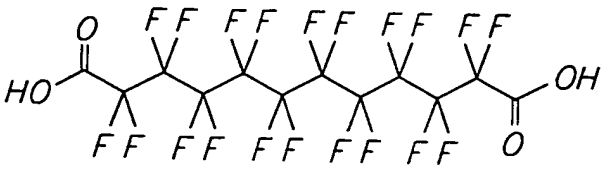
X-94



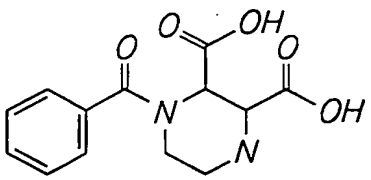
X-95



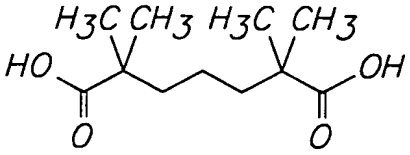
X-96



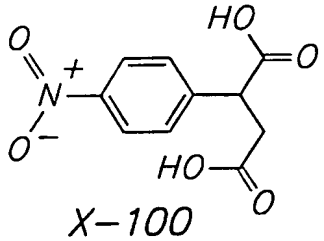
X-97



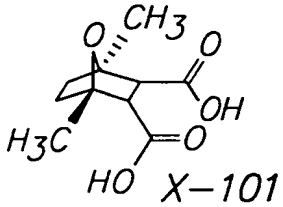
X-98



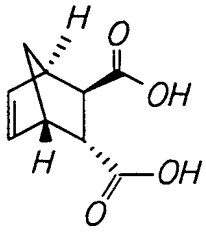
X-99



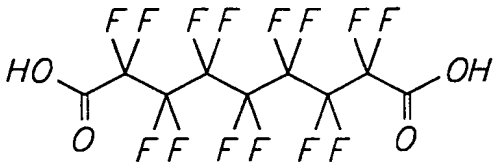
X-100



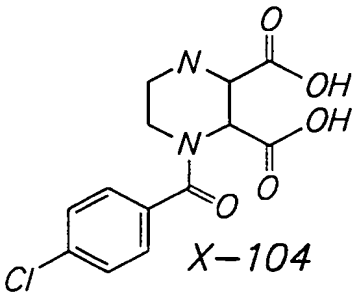
X-101



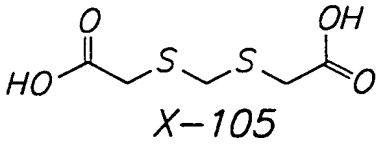
X-102



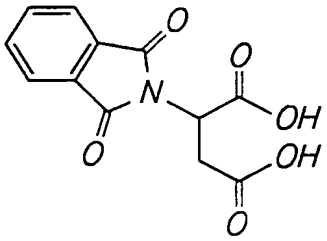
X-103



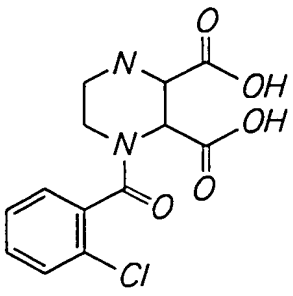
X-104



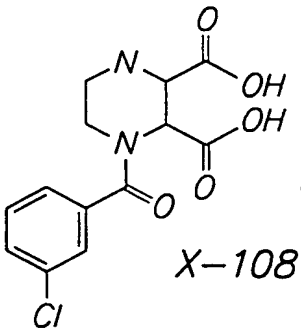
X-105



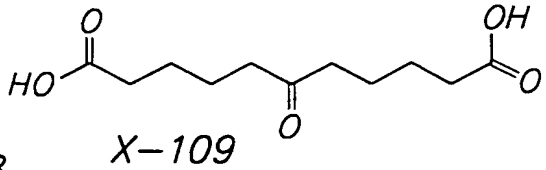
X-106



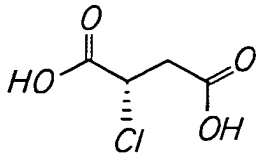
X-107



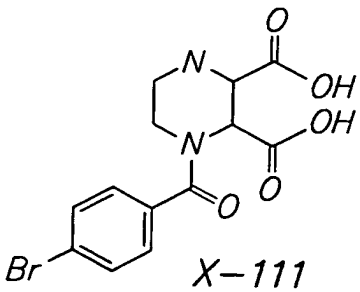
X-108



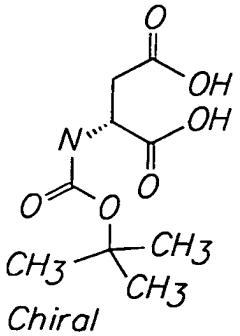
X-109



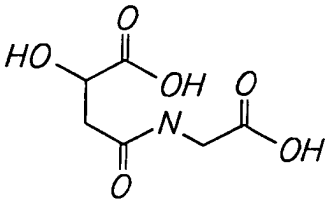
Chiral
X–110



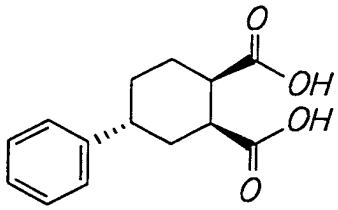
X–111



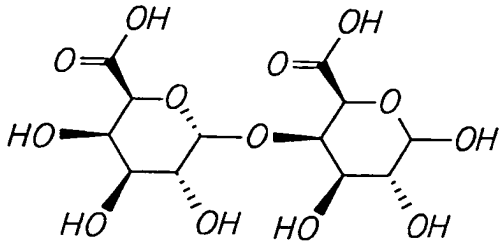
Chiral
X–112



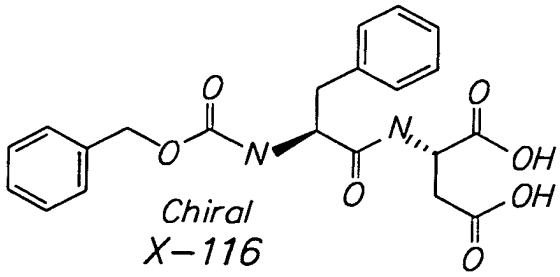
X–113



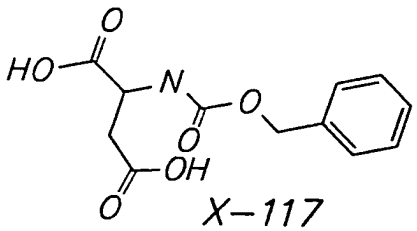
X–114



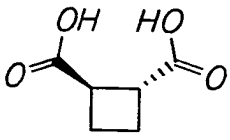
Chiral
X–115



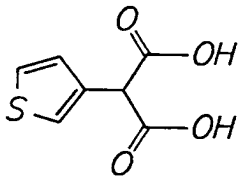
Chiral
X–116



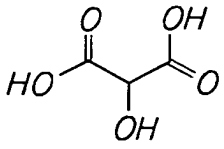
X–117



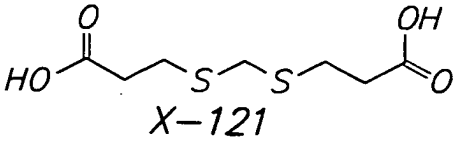
X–118



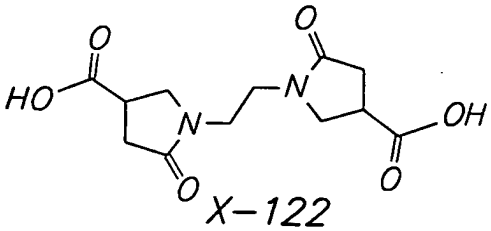
X–119



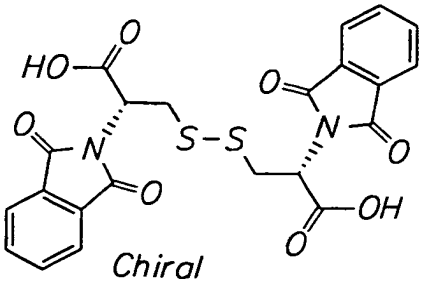
X–120



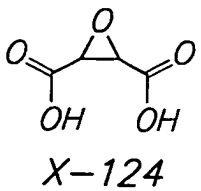
X–121



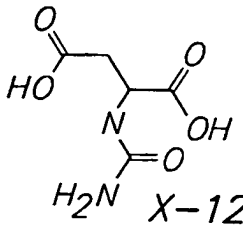
X–122



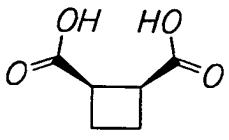
Chiral
X–123



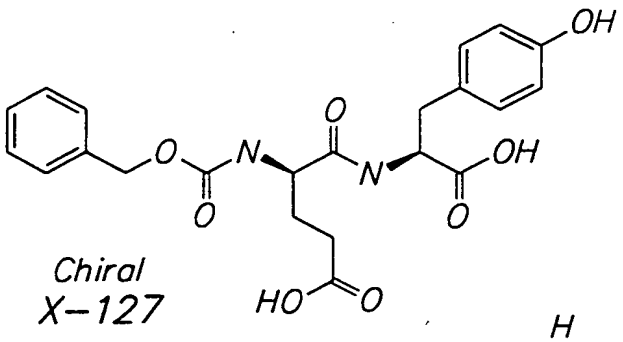
X–124



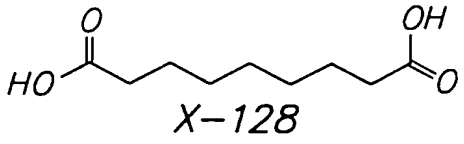
X–125



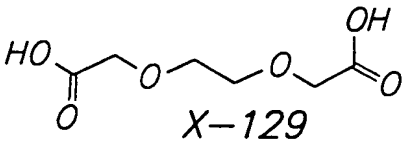
X–126



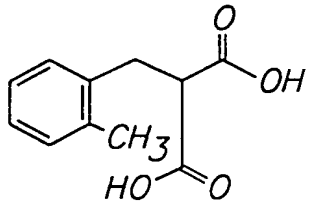
Chiral
X-127



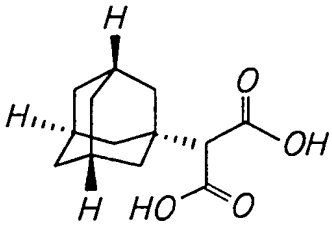
X-128



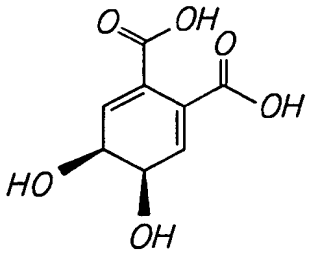
X-129



X-130

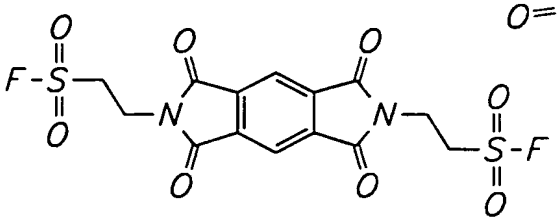


X-131

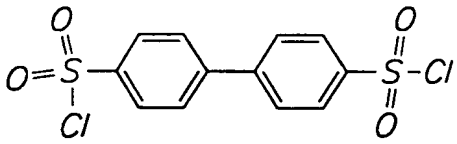


X-132

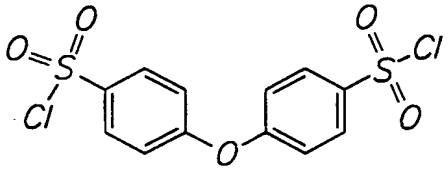
Disulfonyl Halides



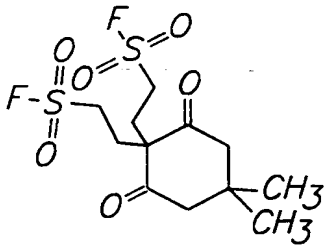
X-133



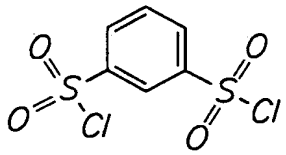
X-134



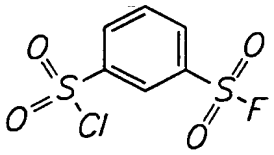
X-135



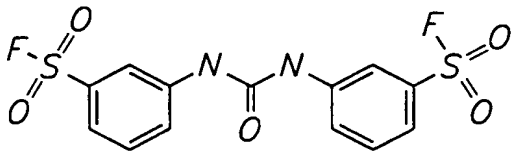
X-136



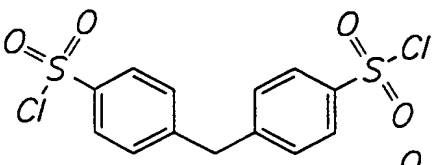
X-137



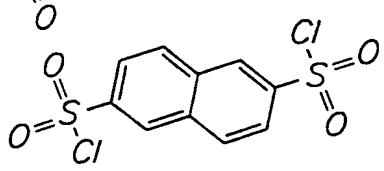
X-138



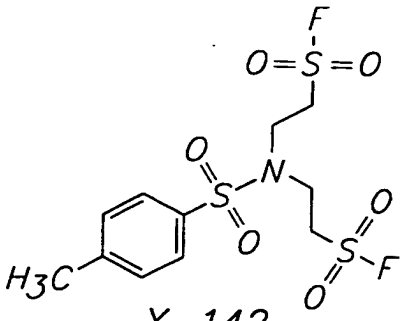
X-139



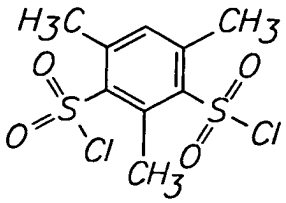
X-140



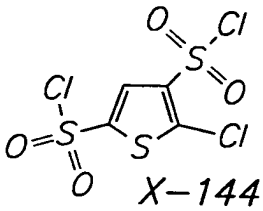
X-141



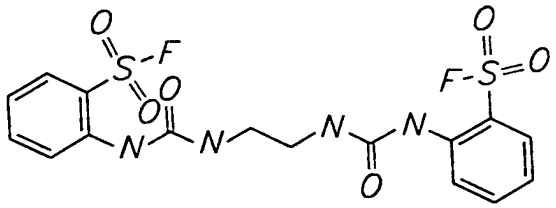
X-142



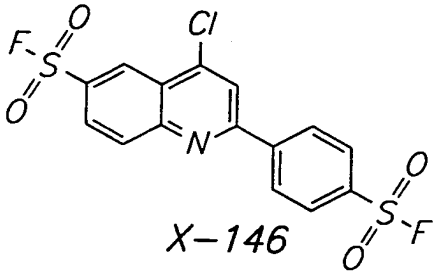
X-143



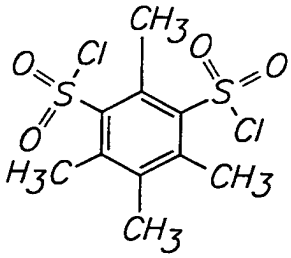
X-144



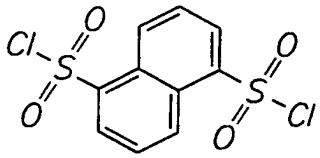
X-145



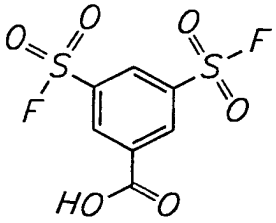
X-146



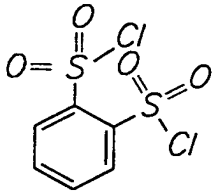
X-147



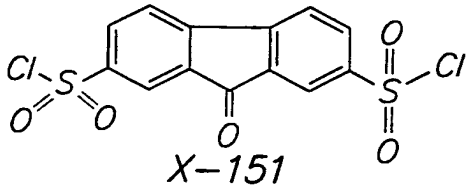
X-148



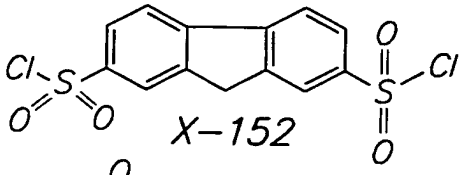
X-149



X-150

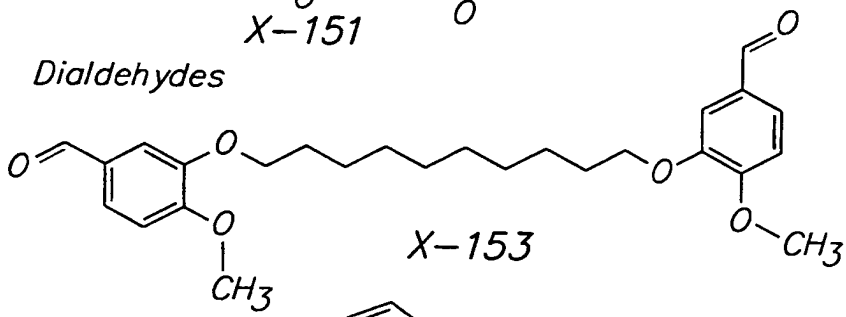


X-151

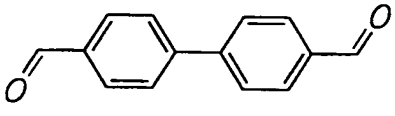


X-152

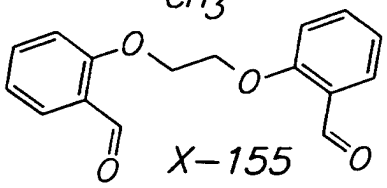
Dialdehydes



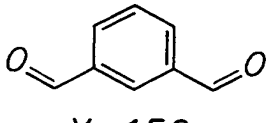
X-153



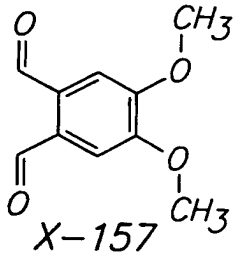
X-154



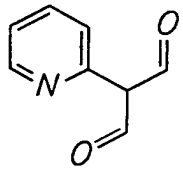
X-155



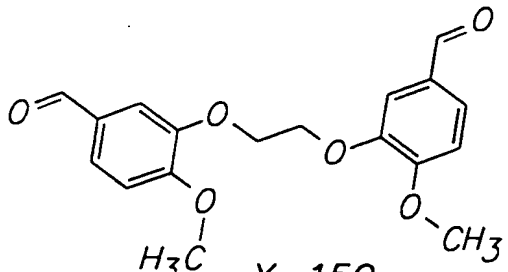
X-156

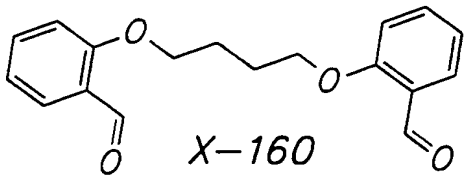


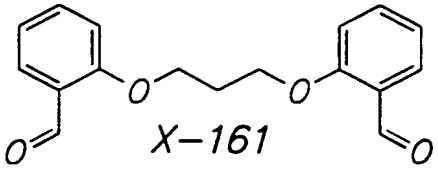
X-157

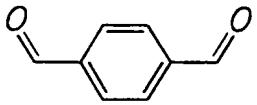


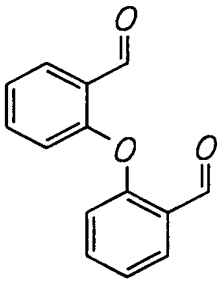
X-158

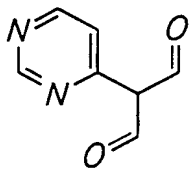

X-159

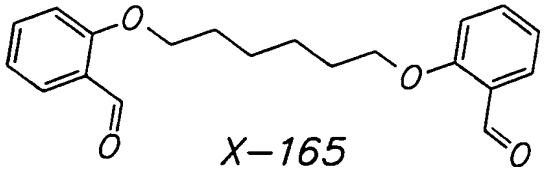

X-160

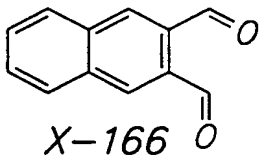

X-161

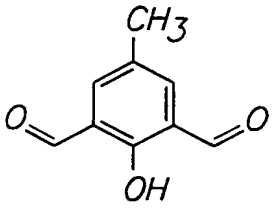

X-162

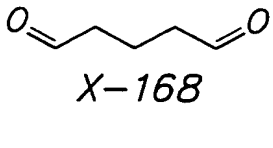

X-163

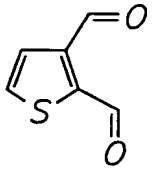

X-164

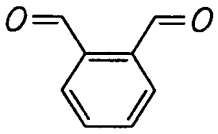

X-165

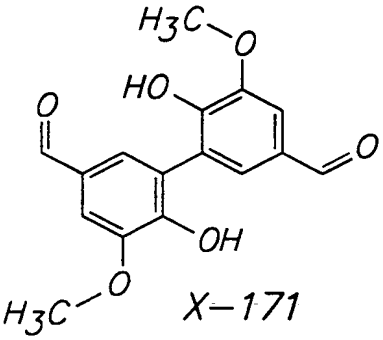

X-166

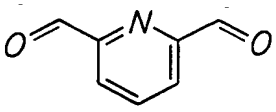

X-167

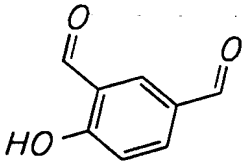

X-168

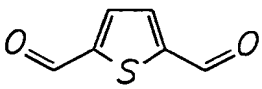

X-169

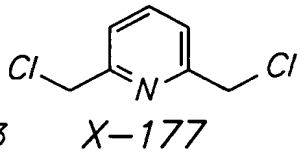

X-170


X-171

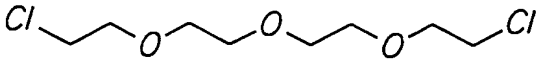

X-172

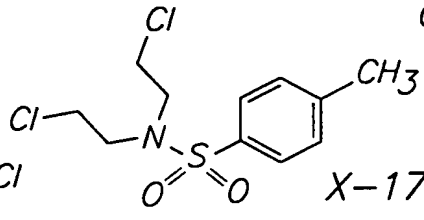

X-173

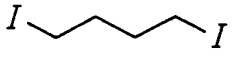

X-174

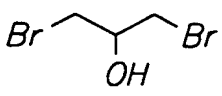

X-177

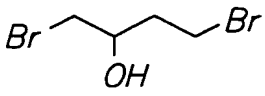
Dihalides

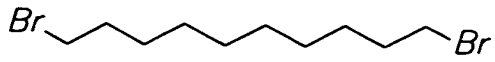

X-175


X-176

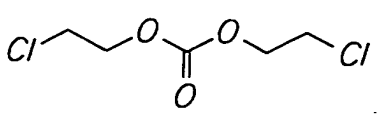

X-178


X-179

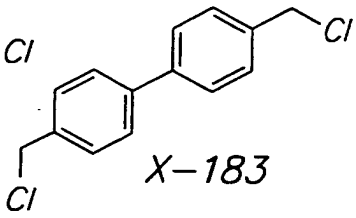

X-180



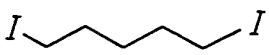
X–181



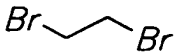
X–182



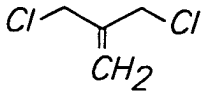
X–183



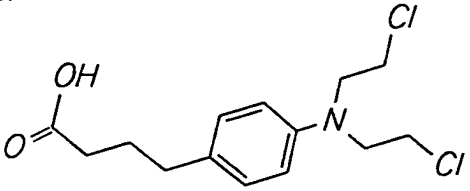
X–184



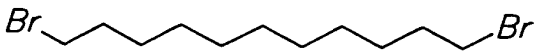
X–185



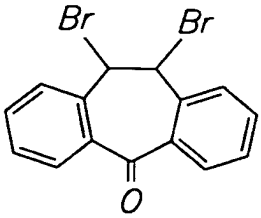
X–186



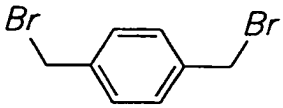
X–188



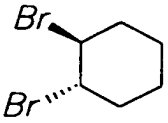
X–187



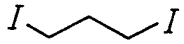
X–190



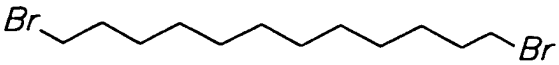
X–189



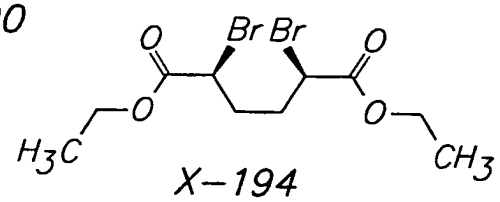
X–191



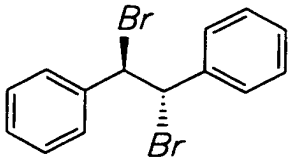
X–192



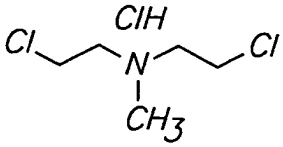
X–193



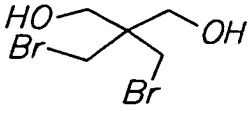
X–194



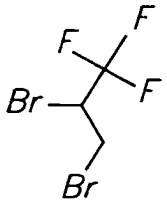
X–195



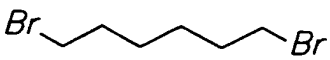
X–196



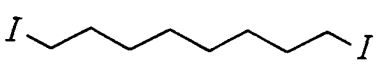
X–197



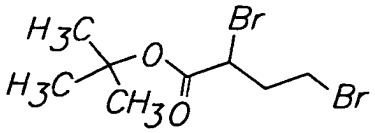
X–198



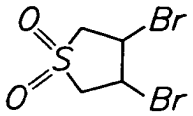
X–199



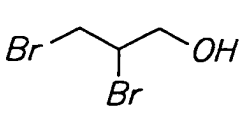
X–200



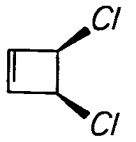
X–201



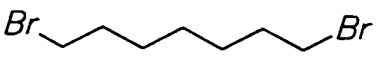
X–202



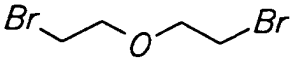
X–203



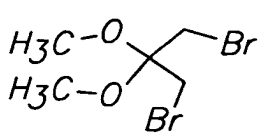
X–204



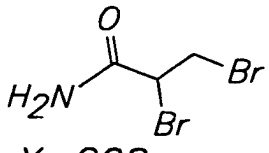
X–205



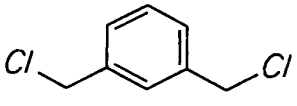
X–206



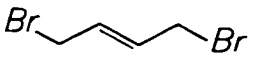
X–207



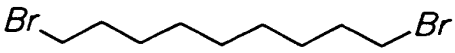
X–208



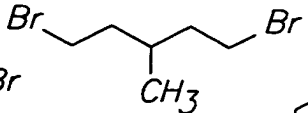
X–209



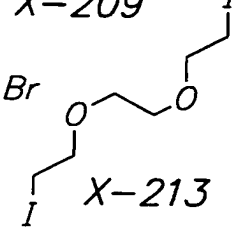
X–210



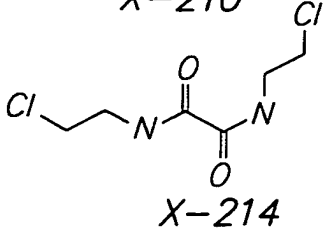
X–211



X–212

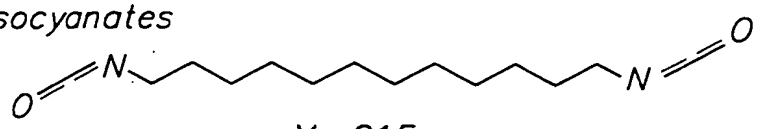


X–213

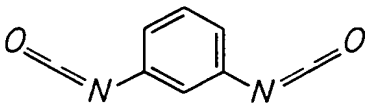


X–214

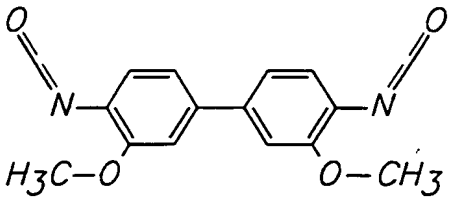
Diisocyanates



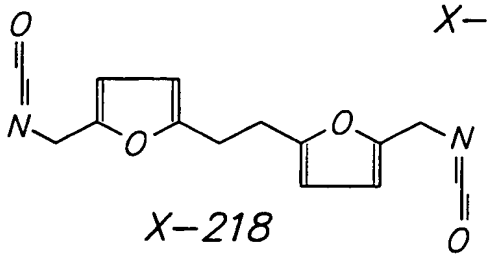
X–215



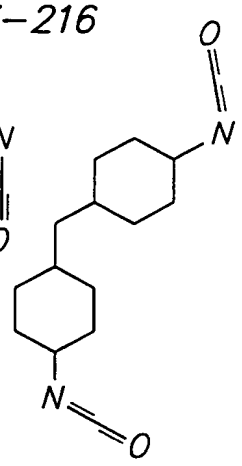
X–216



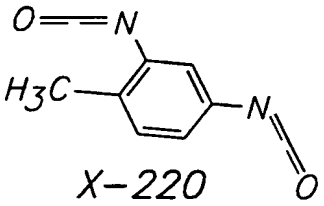
X–217



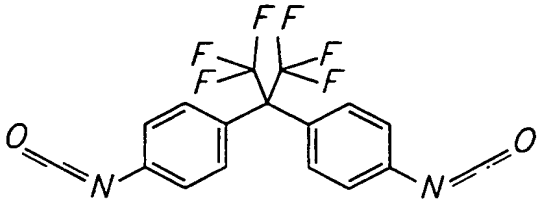
X–218



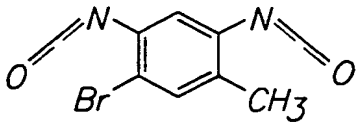
X–219



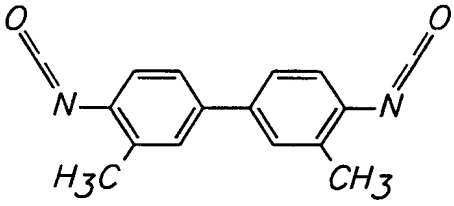
X–220



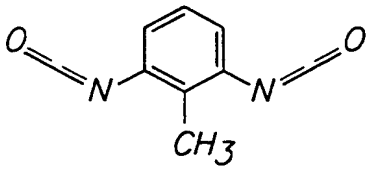
X–221



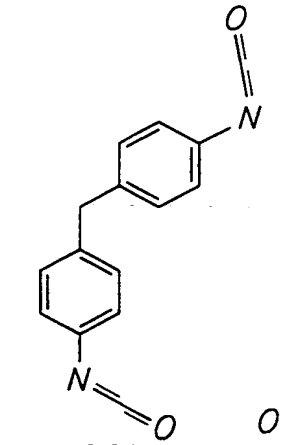
X–222



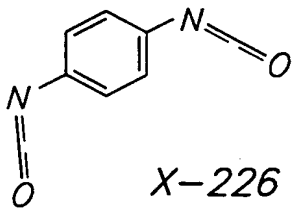
X–223



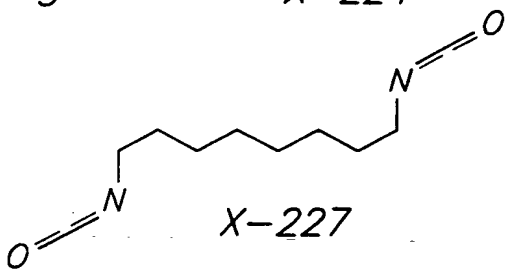
X–224



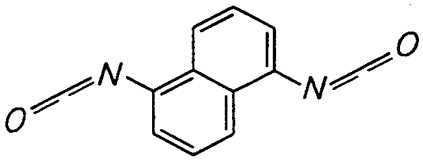
X–225



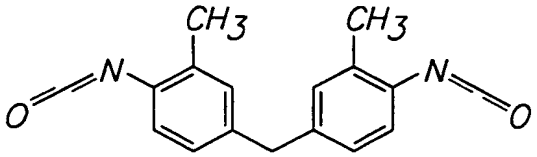
X–226



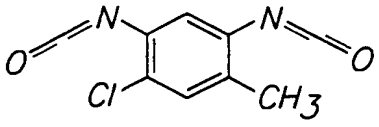
X–227



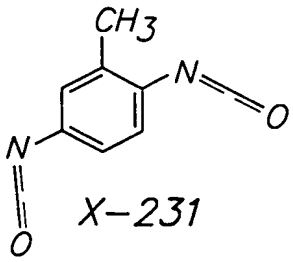
X–228



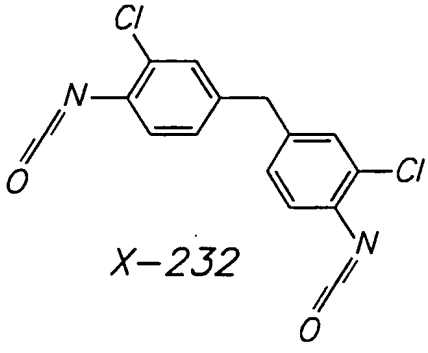
X–229



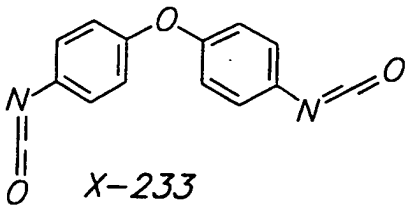
X–230

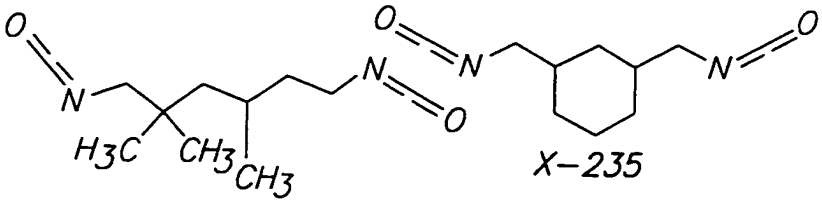


X–231

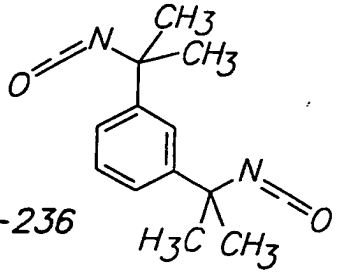


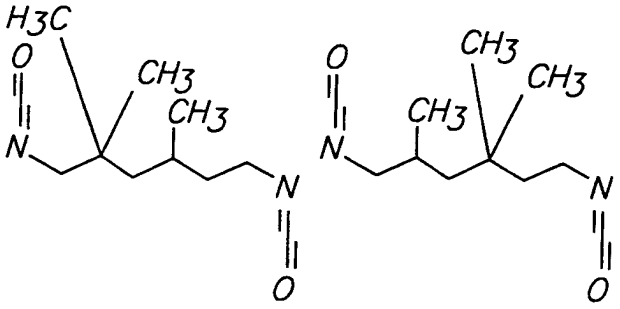
X–232

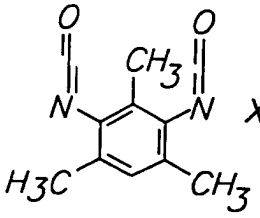

X-233

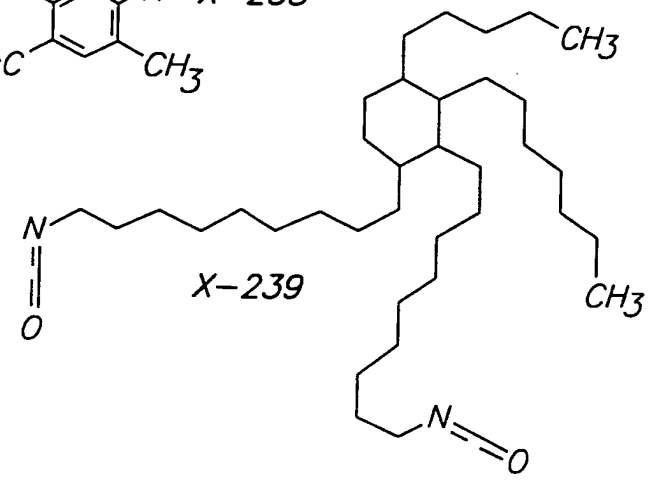

X-235

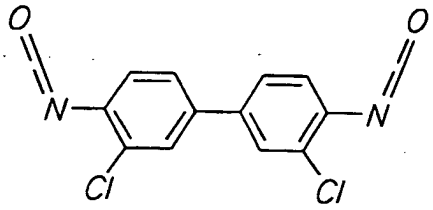
X-234

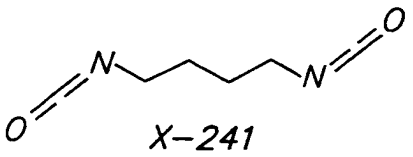

X-236

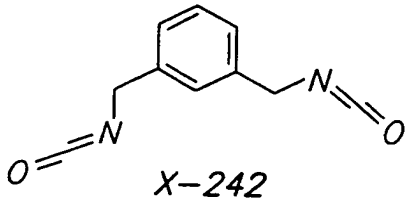

X-237

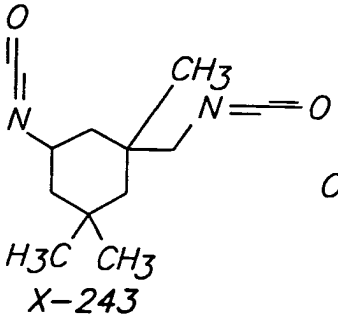

X-238

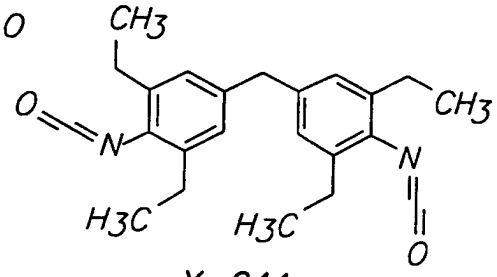

X-239

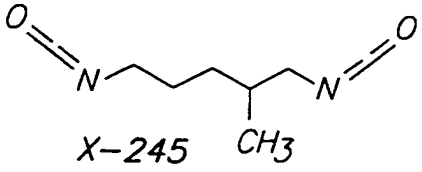

X-240

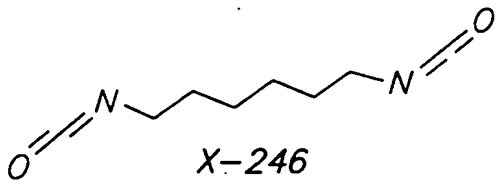

X-241

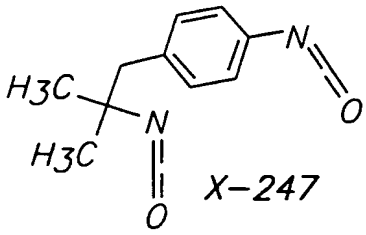

X-242

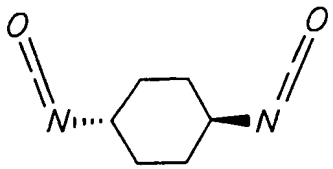

X-243


X-244

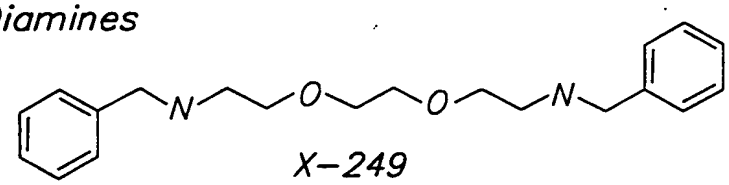

X-245

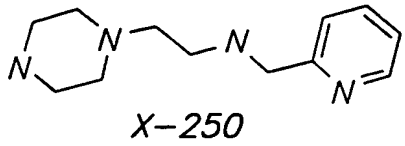

X-246

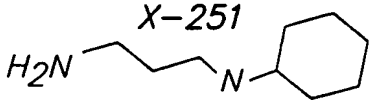

X-247

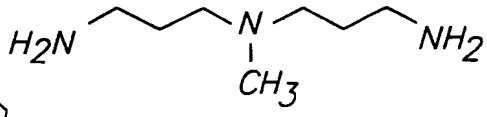

X-248

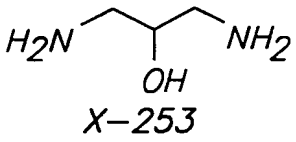
Diamines

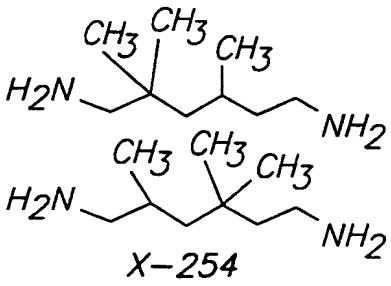

X-249

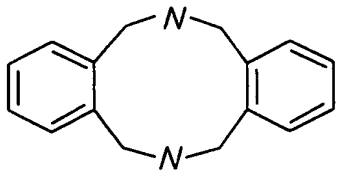

X-250

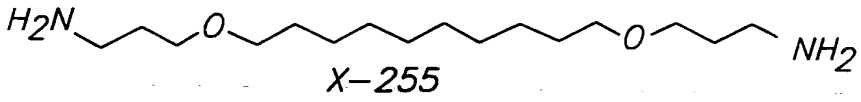

X-251

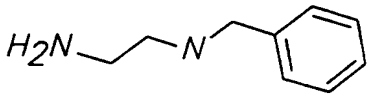

X-252

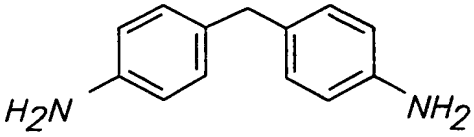

X-253

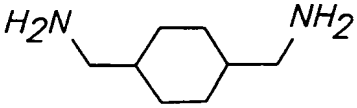

X-254

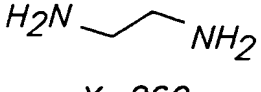

X-256

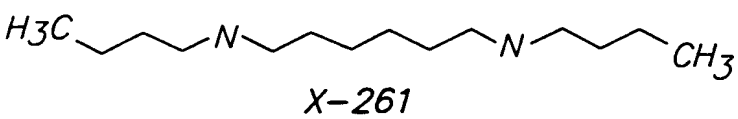

X-255

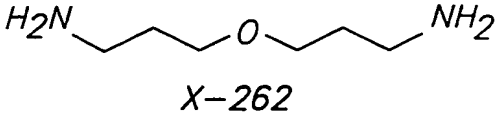

X-257

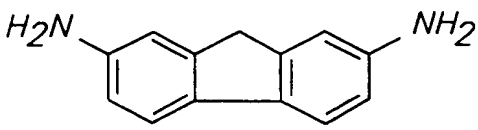

X-258

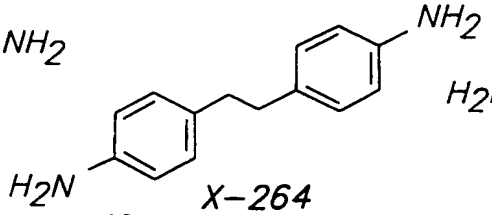

X-259

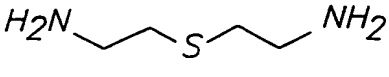

X-260

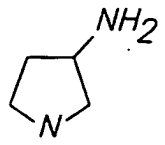

X-261


X-262

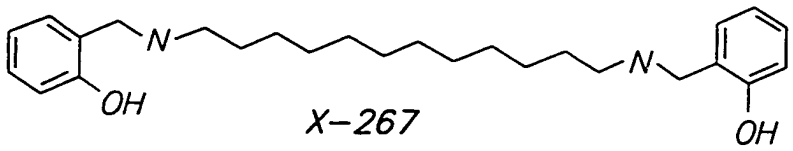

X-263


X-264

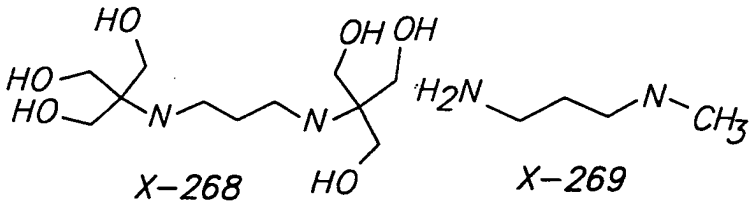

X-265



X-266

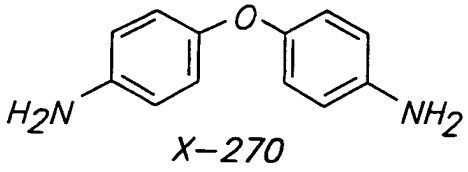


X-267

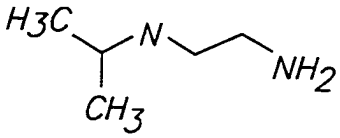


X-268

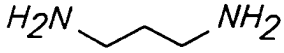
X-269



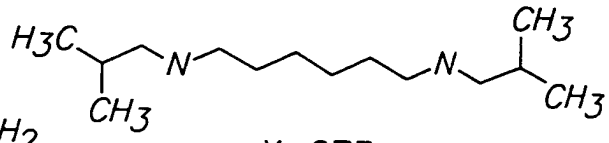
X-270



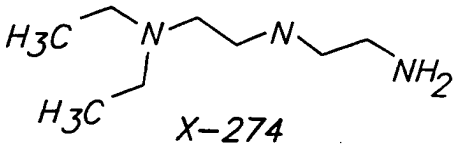
X-271



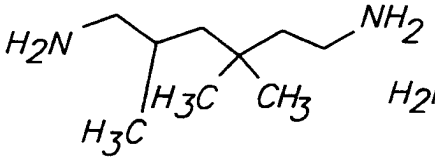
X-272



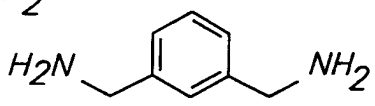
X-273



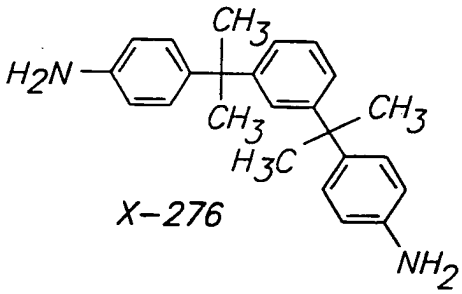
X-274



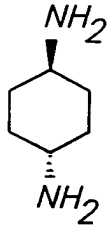
X-275



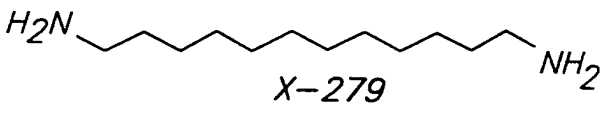
X-277



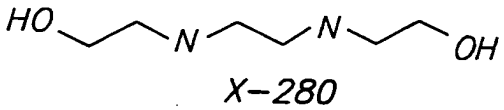
X-276



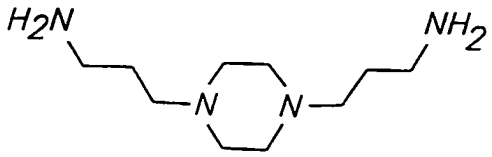
X-278



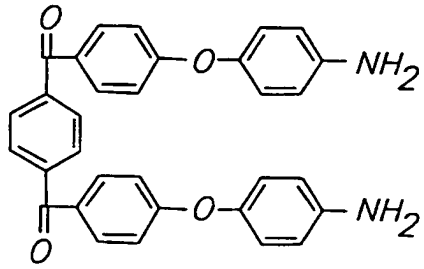
X-279



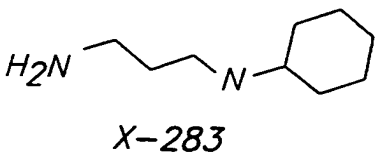
X-280



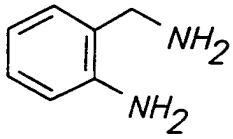
X-281



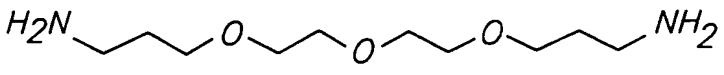
X-282



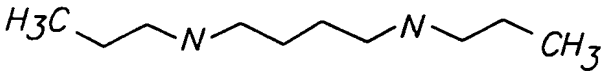
X-283



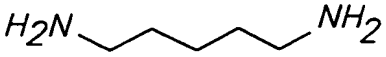
X-284



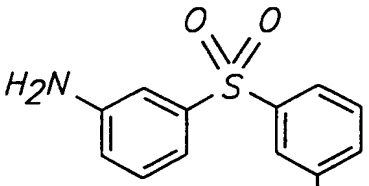
X-285



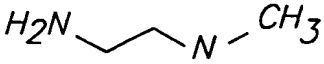
X-286



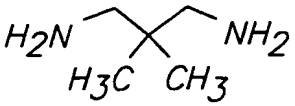
X-287



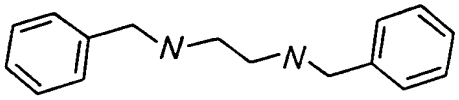
X-288



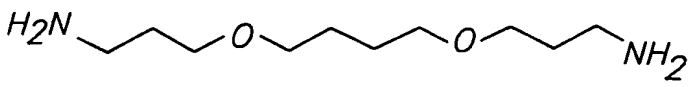
X-289



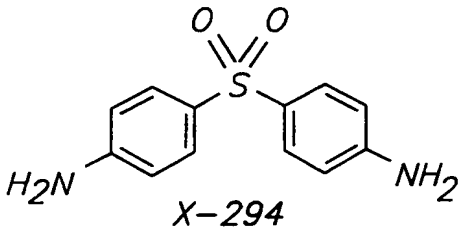
X-290



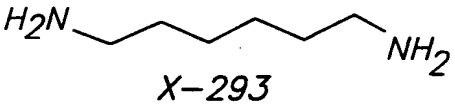
X-292



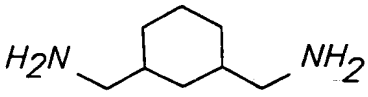
X-291



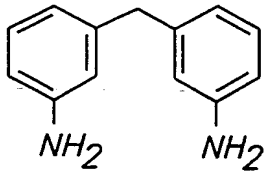
X-294



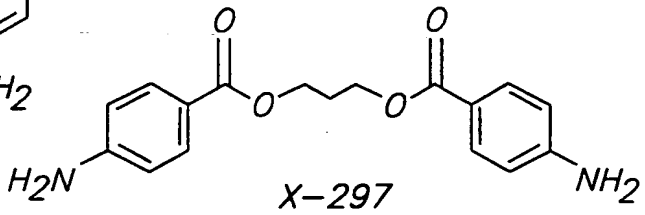
X-293



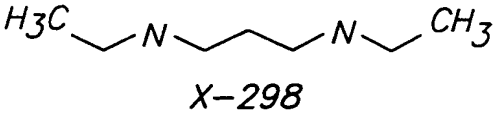
X-295



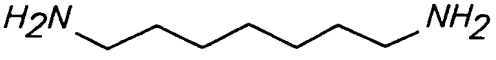
X-296



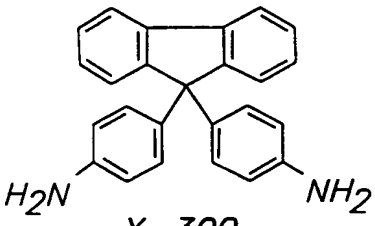
X-297



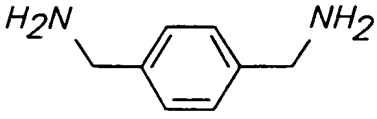
X-298



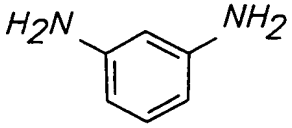
X-299



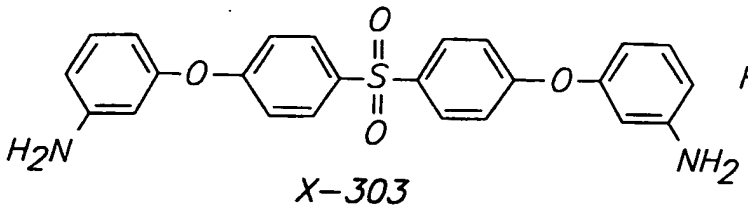
X-300



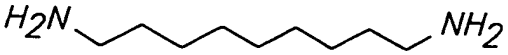
X-301



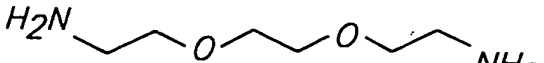
X-302



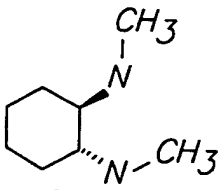
X-303



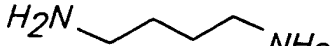
X-304



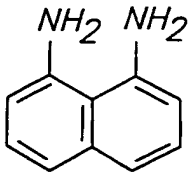
X-305



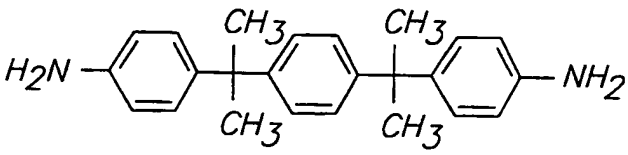
Chiral
X-306



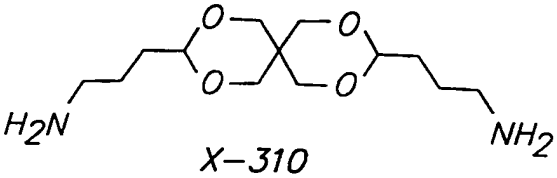
X-307



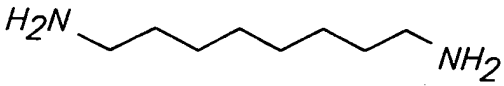
X-308



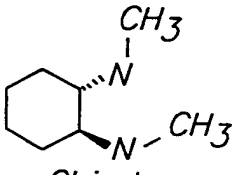
X-309



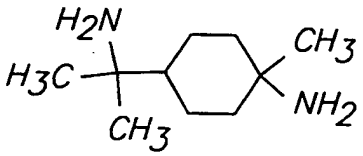
X-310



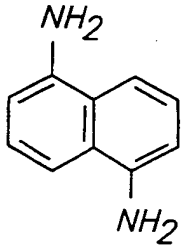
X-311



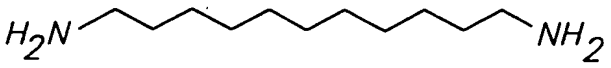
Chiral
X-312



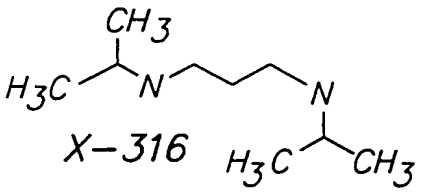
X-313



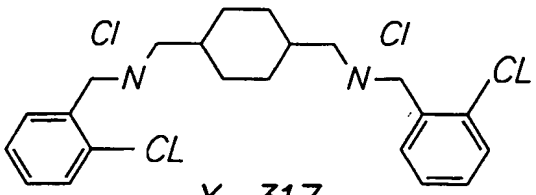
X-314



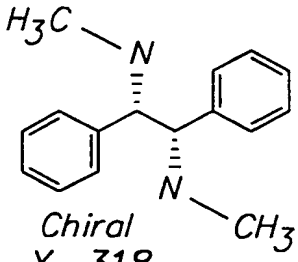
X-315



X-316

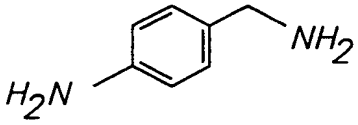


X-317

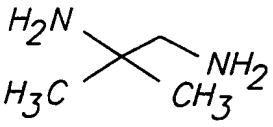


Chiral

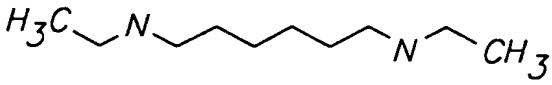
X-318



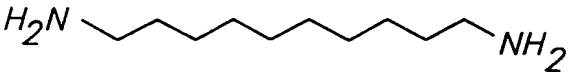
X-319



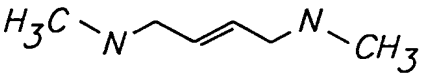
X-320



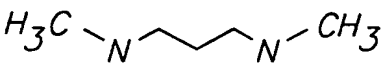
X-321



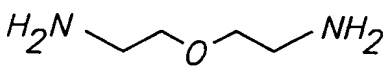
X-322



X-323

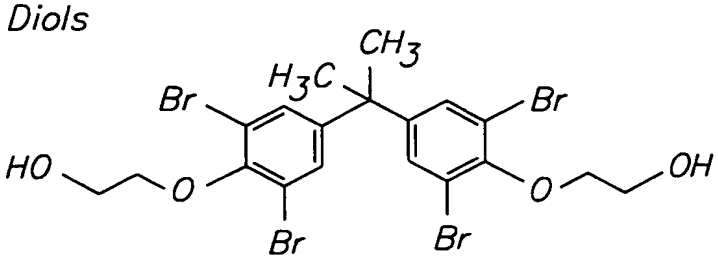


X-324

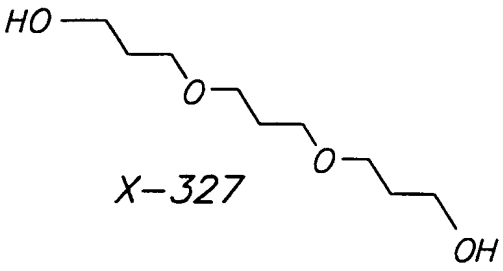


X-325

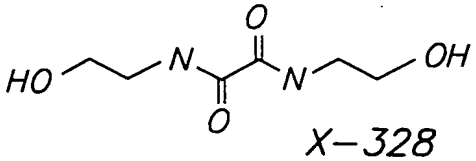
Diols



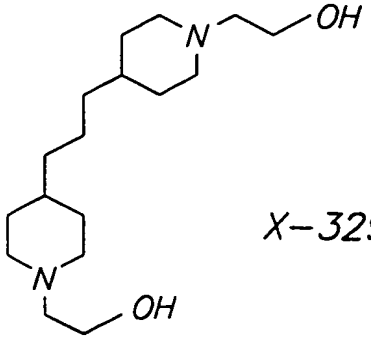
X-326



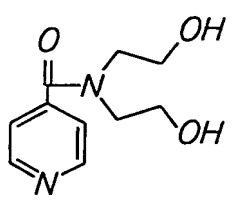
X-327



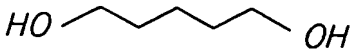
X-328



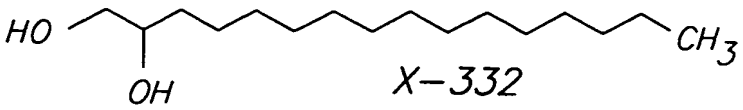
X-329



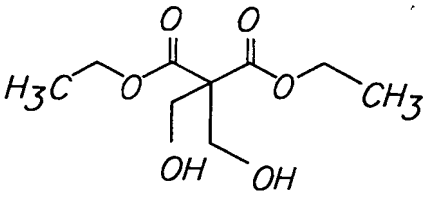
X-330



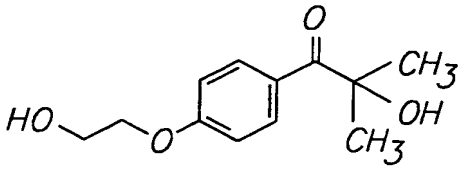
X-331



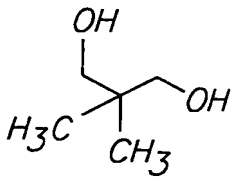
X-332



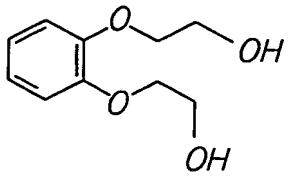
X-333



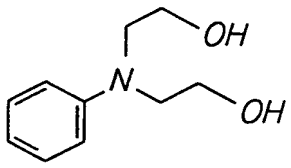
X-334



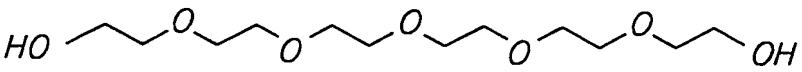
X-335



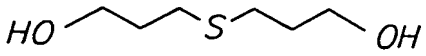
X-336



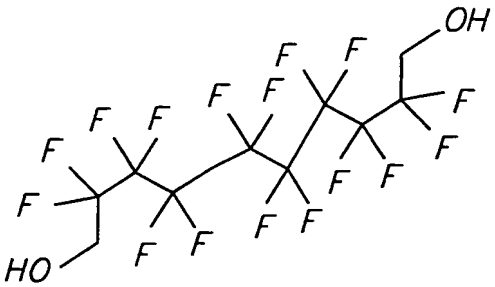
X-337



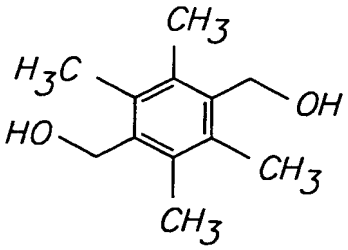
X-338



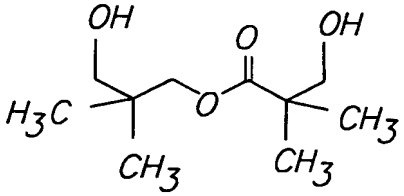
X-339



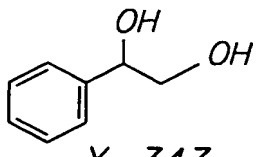
X-340



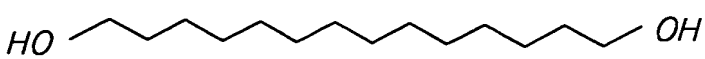
X-341



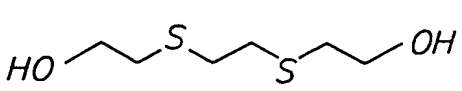
X-342



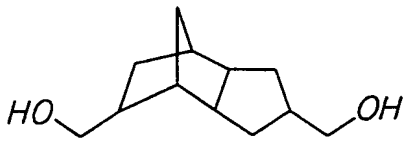
X-343



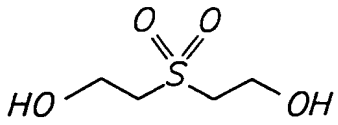
X-344



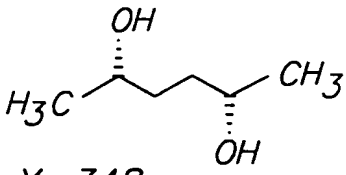
X-345



X-346

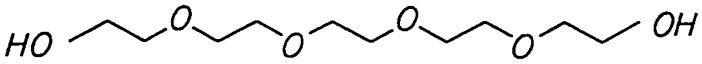


X-347

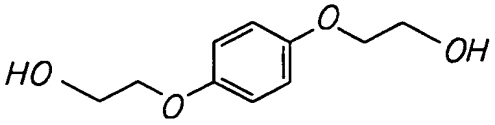


X-348

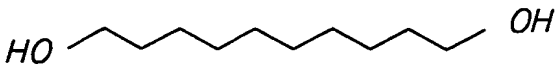
X-349



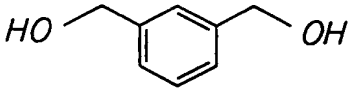
X-350



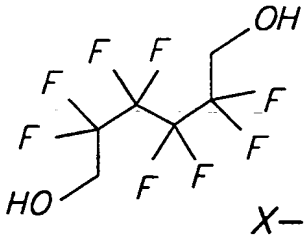
X-351



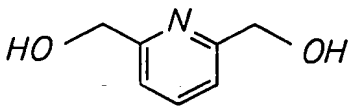
X-352



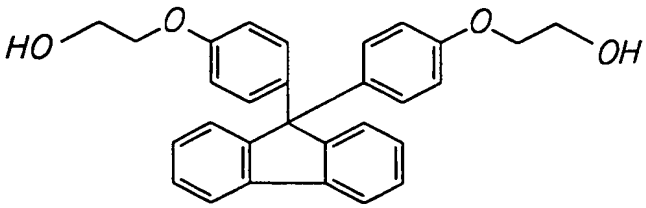
X-353



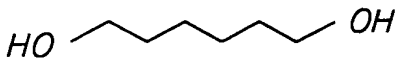
X-354



X-355

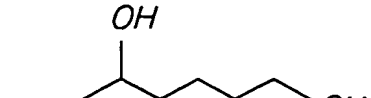


X-356

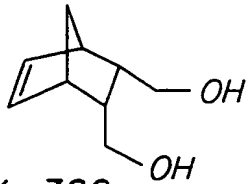


X-357

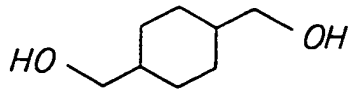
X-358



X-359

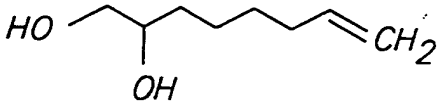


X-360

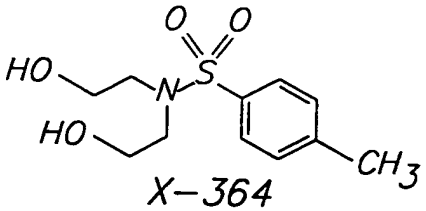


X-361

X-362

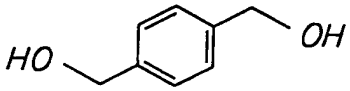


X-363

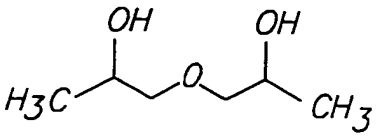


X-364

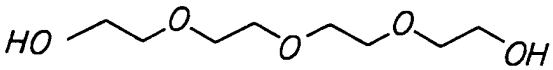
X-365



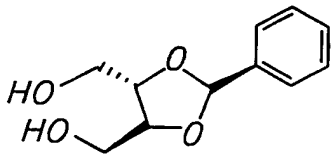
X-366



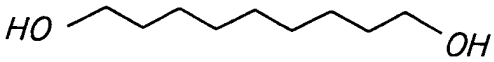
X-367



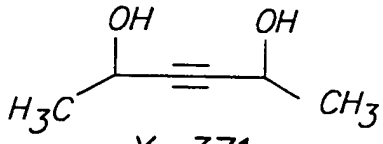
X-368



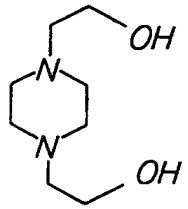
X-369



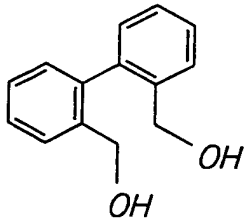
X-370



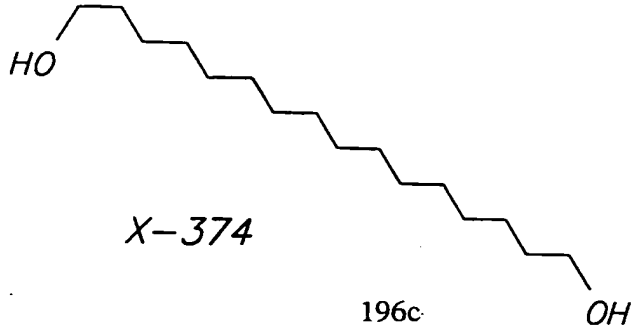
X-371



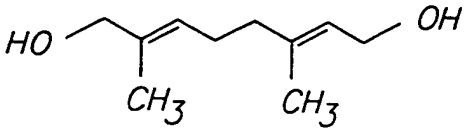
X-372



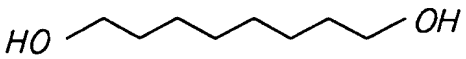
X-373



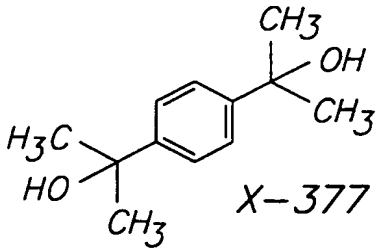
X-374



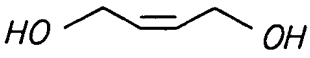
X-375



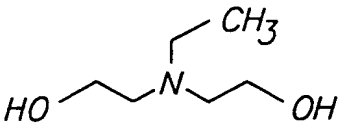
X-376



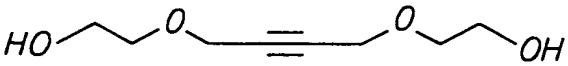
X-377



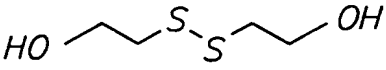
X-378



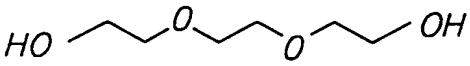
X-379



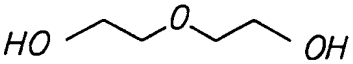
X-380



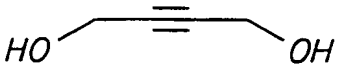
X-381



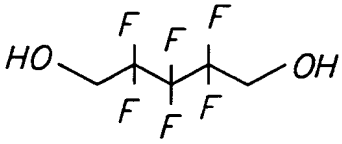
X-382



X-383

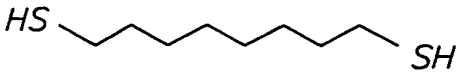


X-384

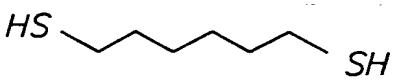


X-385

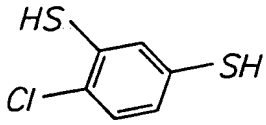
Dithiols



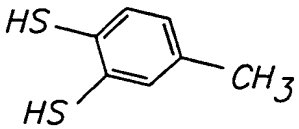
X-386



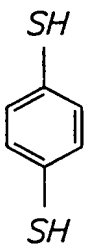
X-387



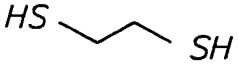
X-388



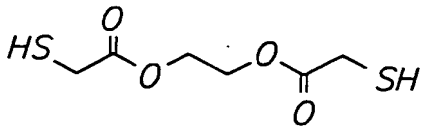
X-389

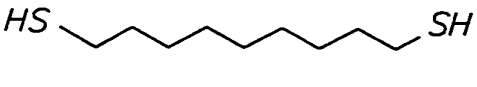


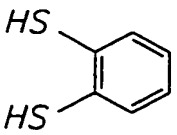
X-390

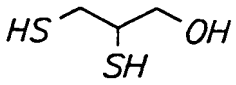


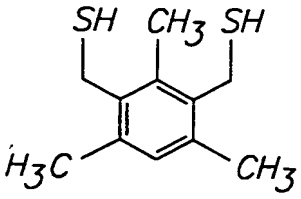
X-391

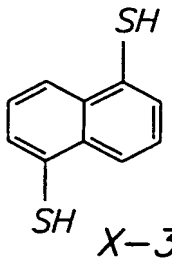

X-392

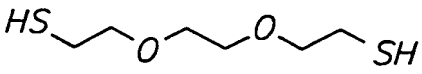

X-393

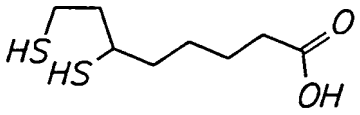

X-394

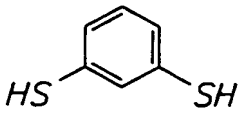

X-395

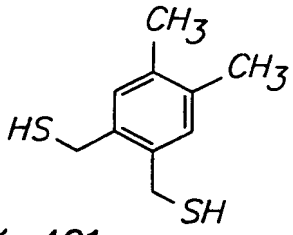

X-396

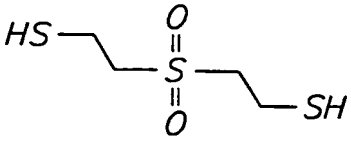

X-397

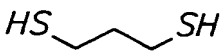

X-398

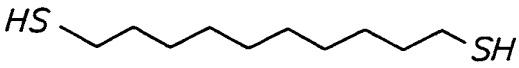

X-399

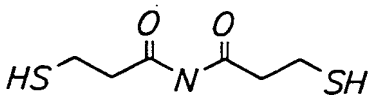

X-400

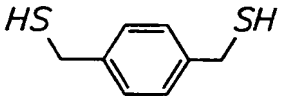

X-401

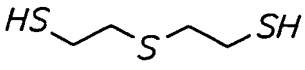

X-402

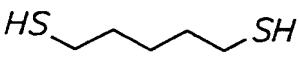

X-403

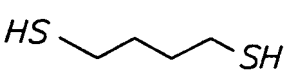

X-404

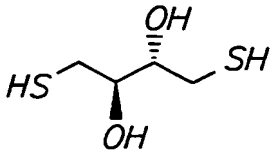

X-405

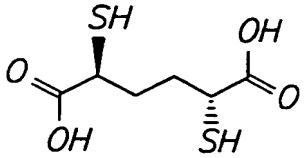

X-406

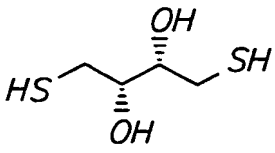

X-407

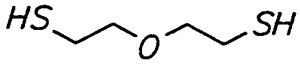

X-408

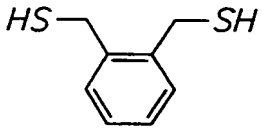

X-409

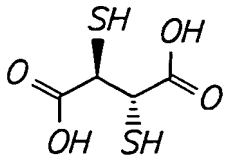

X-410

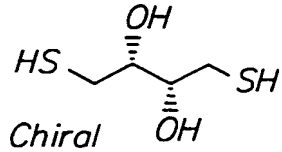

X-411

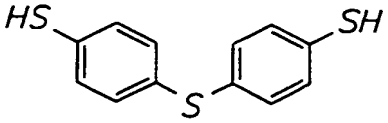

X-412

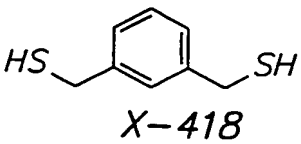

X-413


X-414


X-415


Chiral
X-416


X-417


X-418

IT IS CLAIMED:

1. A multibinding compound of the formula:

$$(L)_pX_q$$

Formula I

in which L is a ligand that may be the same or different at each occurrence;
X is a linker that may be the same or different at each occurrence;
p is an integer of 2-10; and
q is an integer of 1-20,
each of said ligands compromising a ligand domain capable of binding to an enzyme, enzyme substrate, or enzyme cofactors;
or a pharmceutically acceptable salt thereof;
with the proviso that when p is 2 and q is 1 then:
when the enzyme is acrosin, the ligand cannot be a benzamidine;
when the enzyme is a bacterial transglycosylase, the ligand cannot be a glycopeptide;
when the enzyme is bacterial DNA gyrase, the ligand cannot be a quinolone;
when the enzyme is thrombin, the ligand cannot be hirudin analogs;
when the enzyme is thromboxane synthase, the ligand cannot be dazoxiben or isbogrel;
when the enzyme is phosphokinase, the ligand cannot be a cinnamyl derivative;
when the enzyme is oxidoreductase, the ligand cannot be 5-aminosalicylic acid;
when the enzyme is cholesterol o-acyltransferase, the ligand cannot be a furochromone, a furobenzoxazine, or a benzodifuran;
and also with the proviso that;
the enzyme is not a phosphodiesterase or a penicillin binding protein;
the ligand is not cyclosporin, FK506, rapamycin, or rifamycin, or AZT in combination with DDI.

2. The multibinding compound of claim 1, wherein p is 2 and q is 1.

3. The multibinding compound of claim 2, wherein the ligands are different.

4. The multibinding compound of claim 1, wherein the linker or linkers employed are

selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

5. The multibinding compound of claim 4, wherein the linkers are selected to have different linker lengths ranging from about 2 to 100 angstroms.

6. The multibinding compound of claim 5 wherein the linkers are selected to have different linker lengths ranging from about 3 to 40 angstroms.

7. The multibinding compound of claim 1, wherein the linker is represented by the formula:

$$-X'-Z'-(Y'-Z'')_m-Y''-Z'-X'-$$

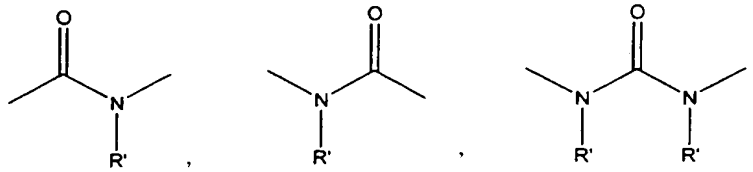
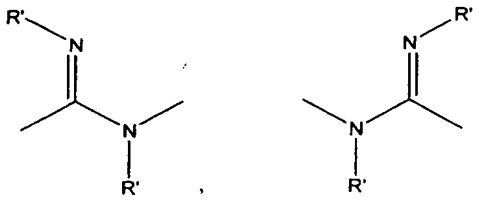
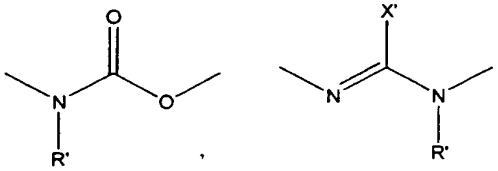
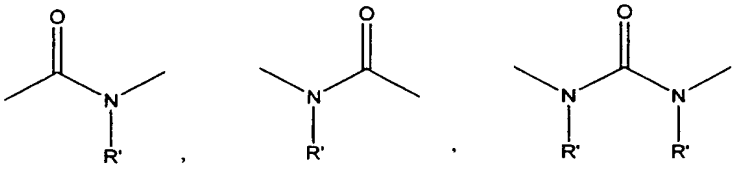
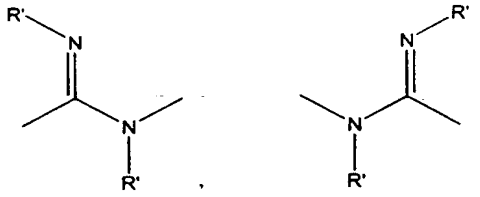
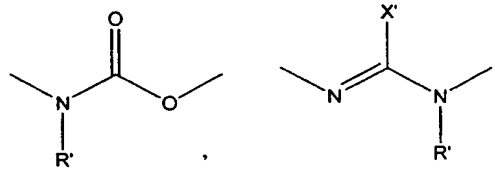
in which:

m is an integer of 0-20;

X' at each separate occurrence is –O-, -S-, -S(O)-, $-S(O)_2-$, –NR- (where R is as defined below), -C(O)-, or a covalent bond;

Z' and Z" at each separate occurrence are alkylene, cycloalkylene, alkenylene, alkynylene, arylene, heteroarylene, heterocycloalkylene, or a covalent bond;

Y' and Y" at each separate occurrence are:



-P(O)$_2$(OR')-O-

-S(O)$_n$-CR'R''-, -S(O)$_n$-NR'-,


-P(O)$_2$(OR')-O-

-S(O)$_n$-CR'R''-, -S(O)$_n$-NR'-,

-O-Z'-O-, -N(R)-Z'-N(R), -S-S-, or a covalent bond; in which:

n is 0, 1 or 2; and

R, R' and R" at each separate occurrence are chosen from hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, and heterocyclo.

8. The multibinding compound of claim 2, wherein the ligands are the same.

9. The multibinding compound of claim 8, wherein the two ligands are covalently linked to the linker at the same point of the ligand.

10. The multibinding compound of claim 9, wherein the two ligands are covalently linked to the linker at different points on the ligand.

11. A pharmaceutical composition comprising a therapeutically effective amount of a multibinding compound of claim 1, or a pharmaceutically acceptable salt thereof; admixed with at least one pharmaceutically acceptable excipient.

12. A method for treating a disease or condition in a mammalian or avian subject which is alleviated by treatment with a multibinding agent, comprising administering to a subject in need of such treatment a therapeutically effective amount of a multibinding compound of claim 1, or a pharmaceutically acceptable salt thereof.

13. The method of claim 12, wherein the method is chosen from treatment of a mammal afflicted with pathogenic bacteria, psoriasis, multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes, breast cancer and prostate cancer, disease states related to blood clotting, Parkinson's disease, transplant rejection, T-cell leukemia, or lymphoma, arthritis, metastatic cancer, tumor growth related to metalloprotease enzymes, stress and hypertension.

14. A method for identifying multimeric ligand compounds that possess multibinding properties with respect to enzymes, which method comprises:

(a) identifying a ligand or mixture of ligands capable of binding to an enzyme and having at least one chemically reactive functional group;

(b) identifying a library of linkers wherein each linker in said library comprises at least two

functional groups having complementary chemical activity to at least one of the ligand functional groups;

(c) preparing a multimeric ligand compound by combining the ligand or ligands identified in step (a) with the library of linkers identified in step (b) under conditions sufficient to form covalent linkages between the complementary functional groups of the ligand or ligands and the linker;and

(d) assaying the multimeric ligand compound library produced in step (c) to identify multimeric ligand compounds that possess multibinding properties.

15. A method for identifying multimeric ligand compounds that possess multibinding properties with respect to enzymes, which method comprises:

(e) identifying a ligand or mixture of ligands capable of binding to an enzyme and having at least one chemically reactive functional group;

(f) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary chemical activity to at least one of the ligand functional groups;

(g) preparing a multimeric ligand compound by combining the ligand or ligands identified in step (a) with the library of linkers identified in step (b) under conditions sufficient to form covalent linkages between the complementary functional groups of the ligand or ligands and the linker;and

(h) assaying the multimeric ligand compound library produced in step (c) to identify multimeric ligand compounds that possess multibinding properties.

16. The method of claim 14 or 15, wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

17. The method of claim 16, wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

18. The method of claim 17, wherein each member of the library is isolated and identified by

preparative liquid chromatography mass spectrometry (LCMS).

19. The method of claim 18, wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

20. The method of claim 19, wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

21. The method of claim 20, wherein the linkers are selected to have different linker lengths ranging from about 2 to 100 angstroms.

22. The method of claims 14 or 15, wherein said reactive functional groups of ligands is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof, wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

23. A library of multimeric ligand compounds which may possess multivalent properties, which library is prepared by the method comprising:

(a) identifying a library of ligands, wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

24. The library of claim 23, wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

25. The library of claim 24, wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

26. The library of claim 25, wherein the linkers are selected to have different linker lengths ranging from about 2 to 100 angstroms.

27. The library of claim 23, wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

28. The library of claim 27, wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

29. The library of claim 23, wherein the multimeric ligand compound library comprises homomeric ligand compounds.

30. The library of claim 23, wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

31. An iterative method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of a ligand or mixture of ligands

which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

32. The method of claim 31, wherein steps (e) and (f) are repeated from 2-50 times.

33. The method of claim 32, wherein steps (e) and (f) are repeated from 5-50 times.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12620

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	COLE et al. Discovery of Chiral Catalysts through Ligand Diversity: Ti-Catalyzed Enantioselective Addition of TMSCN to *meso* Epoxides. Angew. Chem. Int. Ed. Engl. 1996, Vol. 35, No. 15, pages 1668-1671, see pages 1669-1670, Figure 1 and Scheme 2.	23-28, 30 ---- 1-22, 29, 31-33
X --- Y	MENGER et al. Phosphatase Catalysis Developed *via* Combinatorial Organic Chemistry. J. Org. Chem. 1995, Vol. 60, pages 6666-6667, see entire article.	23-28, 30 ---- 1-22, 29, 31-33

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
10 SEPTEMBER 1999	25 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MAURIE E. GARCIA Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12620

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	HOLMES et al. Synthesis and Structure-Activity Relationships of a Series of Penicillin-Derived HIV Proteinase Inhibitors Containing a Stereochemically Unique Peptide Isostere. J. Med. Chem. October 1993, Vol. 36, No. 21, pages 3129-3136, see entire document.	1-6, 8-10 ---- 7, 11-33
X --- Y	VELAZQUEZ et al. Synthesis and Anti-HIV Activity of [AZT]-[TSAO-T] and [AZT]-[HEPT] Dimers as Potential Multifunctional Inhibitors of HIV-1 Reverse Transcriptase. J. Med. Chem. 12 May 1995, Vol. 38, No. 10, pages 1641-1649, see entire document, especially Abstract, Chart 1, Schemes 1-3 and Tables 2-3.	1-6 ---- 7-33
X --- Y	US 5,519,134 A (ACEVEDO et al) 21 May 1996 (21/05/96), see Abstract, column 1 lines 21-38, column 3 lines 20-34, column 6 lines 19-67, column 7 lines 1-4 and columns 11-12.	1-6, 8-10, 13-30 ---- 7, 11-14, 31-33
Y	WO 92/05802 A1 (NEORX CORPORATION) 16 April 1992 (16/04/92), see Abstract, page 3 lines 1-25, page 4 lines 20-27, page 5 lines 6-18, page 21 lines 4-33, page 22 lines 1-8 and claim 1.	1-33
Y	WO 97/35195 A1 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 25 September 1997 (25/09/97), see page 3 lines 17-32, page 4 lines 1-18, page 7 lines 26-34, page 8 lines 1-5 and claims 13, 35 & 36.	14-33
Y	SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	14-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12620

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12620

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN (CAPLUS, BIOSIS, SCISEARCH, MEDLINE)
Search Terms: multibinding, multivalent, enzyme, bind?, mevastatin, simvastatin, lovastatin, link? coupling?, coupled, combinatorial, library

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-22, drawn to multibinding compounds, multimeric ligand compounds that possess multibinding properties with respect to enzymes, pharmaceutical compositions, methods of treatment and methods of identifying such compounds.
Group II, claim(s) 23-30, drawn to a library of multimeric ligand compounds.
Group III, claim(s) 31-33, drawn to an iterative method of identifying multimeric ligand compounds.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I has a different special technical feature than Groups II and III. The technical feature of Group I is the compounds that posses multibinding properties with respect to enzymes. The technical feature of Groups II and III is the multimeric ligand compound library. Note that the limitation that these compounds posses properties with respect to enzymes is not present in the claims of Groups II and III.

The technical feature that links the claims in Groups II and III is the multimeric ligand compound library. These groups lack unity because the libraries are known in the art.

For example, Cole et al (Angew. Chem. Int.Ed. Engl., 1996, Vol. 35, No. 15, pp. 1668-1671) teaches "diverse peptide-based structures" that can be collectively screened to search for chiral catalysts (see page 1669, 1st column). These ligands possess at least three sites for binding, as shown in structure 3 (2 hydroxy groups and the nitrogen). Figure 1 of Cole et al (page 1670) shows the variation of the ligand components. The middle amino acid component (AA2) is a linker, with the amino and acid end s comprising two functional groups having complementary reactivity to the other portions of the ligand. Cole et al shows using a library of linkers (choices for AA2 in Figure 1). The other portions are varied as shown in the figure (other amino acid segment and aldehyde segments a-m)and form covalent linkages to AA2 by reaction with the complementary functional groups (see Scheme 2, page 1669).

Additionally, Menger et al (J. Org. Chem., 1995, Vol. 60, pp. 6666-6667) teaches a multimeric ligand compound library comprising a single linker molecule. the linker is poly allylamine, which has a multitude of amino groups that are complementary to the library of ligands having a reactive carboxylic acid functionality. The library of ligands are covalently attached to the linker polymer, this is shown in Figure 1 (page 6666).